BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

```
    (c) 2002 The Dialog Corporation

    Set  Items  Description
    ---  -----  -----------
? set hi %%%;set hi %%%
HILIGHT set on as '%%%'%%%
%%%HILIGHT set on as '%%%'
? b 411
     20oct02 09:01:13 User217743 Session D572.2
        $0.00    0.070 DialUnits File410
    $0.00  Estimated cost File410
    $0.01  TELNET
    $0.01  Estimated cost this search
    $0.01  Estimated total session cost   0.244 DialUnits
File 411:DIALINDEX(R)

DIALINDEX(R)
  (c) 2002 The Dialog Corporation plc

*** DIALINDEX search results display in an abbreviated
*** *** format unless you enter the SET DETAIL ON
command. *** ? set files biochem
>>>       162 is unauthorized
>>>1 of the specified files is not available
  You have 22 files in your file list.
  (To see banners, use SHOW FILES command)
? s angiogenic()fusion()protein?

Your SELECT statement is:
  s angiogenic()fusion()protein?

       Items   File
       -----   ----

  No files have one or more items; file list includes 22
files.
? s pleiotropin or hbnf

Your SELECT statement is:
  s pleiotropin or hbnf

       Items   File
       -----   ----
         37    5: Biosis Previews(R)_1969-2002/Oct W2
       20    34: SciSearch(R) Cited Ref
Sci_1990-2002/Oct W3              3    50: CAB
Abstracts_1972-2002/Sep
          1    65: Inside Conferences_1993-2002/Oct W2
          5    71: ELSEVIER BIOBASE_1994-2002/Oct
W2
         20    73: EMBASE_1974-2002/Oct W2
          2    98: General Sci
Abs/Full-Text_1984-2002/Sep            27   144:
Pascal_1973-2002/Oct W2
         24   155: MEDLINE(R)_1966-2002/Oct W2
          1   156: ToxFile_1965-2002/Oct W3
          1   305: Analytical Abstracts_1980-2002/Oct
W1           21   399: CA
SEARCH(R)_1967-2002/UD=13716
          1   434: SciSearch(R) Cited Ref
Sci_1974-1989/Dec
  13 files have one or more items; file list includes 22
files.
? rf
Your last SELECT statement was:
  S PLEIOTROPIN OR HBNF

Ref      Items   File
---      -----   ----
N1          37    5: Biosis Previews(R)_1969-2002/Oct W2
N2          27   144: Pascal_1973-2002/Oct W2
N3          24   155: MEDLINE(R)_1966-2002/Oct W2
N4          21   399: CA
SEARCH(R)_1967-2002/UD=13716
N5          20    34: SciSearch(R) Cited Ref
Sci_1990-2002/Oct W3 N6           20    73:
EMBASE_1974-2002/Oct W2
N7           5    71: ELSEVIER BIOBASE_1994-2002/Oct
W2
N8           3    50: CAB Abstracts_1972-2002/Sep
N9           2    98: General Sci
Abs/Full-Text_1984-2002/Sep N10           1    65: Inside
Conferences_1993-2002/Oct W2    13 files have one or
more items; file list includes 22 files.
      - Enter P or PAGE for more -
? b 155, 5
     20oct02 09:02:12 User217743 Session D572.3
        $1.14    0.650 DialUnits File411
    $1.14  Estimated cost File411
    $0.21  TELNET
    $1.35  Estimated cost this search
    $1.36  Estimated total session cost   0.895 DialUnits
SYSTEM:OS  - DIALOG OneSearch
  File 155:MEDLINE(R)  1966-2002/Oct W2
*File 155: Alert feature enhanced for multiple files,
duplicates removal, customized scheduling. See HELP
ALERT.
  File   5:Biosis Previews(R)  1969-2002/Oct W2
       (c) 2002 BIOSIS
*File   5: Alert feature enhanced for multiple files,
duplicates removal, customized scheduling. See HELP
ALERT.

    Set  Items  Description
    ---  -----  -----------
? s pleiotropin or hbnf
          28  PLEIOTROPIN
          35  HBNF
    S1    61  PLEIOTROPIN OR HBNF
? s s1 and fusion()protein?
          61  S1
      172696  FUSION
     2892396  PROTEIN?
       69000  FUSION(W)PROTEIN?
    S2     2  S1 AND FUSION()PROTEIN?
? rd
```

...completed examining records
S3 2 RD (unique items)
? t s3/3,ab/1,2

3/3,AB/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08094895 94220851 PMID: 8167469
Refolding and characterization of human recombinant heparin-binding neurite-promoting factor.
Seddon A P; Hulmes J D; Decker M M; Kovesdi I; Fairhurst J L; Backer J; Dougher-Vermazen M; Bohlen P
Department of Protein Chemistry, American Cyanamid Company, Lederle Laboratories, Pearl River, New York 10965.
Protein expression and purification (UNITED STATES) Feb 1994, 5 (1) p14-21, ISSN 1046-5928 Journal Code: 9101496
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Heparin-binding neurite-promoting factor (%%%HBNF%%%) is a highly basic, cysteine-rich 136-residue protein, and a member of a new class of heparin-binding proteins. It exhibits a neurite-outgrowth promoting activity and its expression is both temporally and spacially regulated during fetal and postnatal development. A high interspecies sequence conservation suggests important, presently unknown, biological functions. %%%HBNF%%% is structurally and most likely functionally related to the product of a developmentally regulated gene, MK (midkine). To elucidate biological roles of these proteins, recombinant forms of the proteins were produced. Expression of human recombinant %%%HBNF%%% and MK in Escherichia coli lead to the formation of insoluble aggregated protein that accounted for about 25% of the total cellular protein. Homogeneous, monomeric forms of each protein were recovered from inclusion bodies by reduction with dithiothreitol and solubilization in 8 M urea. Refolding of the reduced and denatured protein occurred upon dialysis at pH 7.4. Human recombinant (hr) %%%HBNF%%% and hrMK prepared in this manner were further purified by heparin affinity chromatography. Chromatographic evidence demonstrates that refolding and concomitant disulfide bond formation in hrHBNF proceeds in high yield with minimal formation of stable nonnative disulfides. Studies on the redox status of the 10 cysteine residues of bovine brain %%%HBNF%%% and the refolded recombinant protein indicate that all cysteines are engaged in disulfide bond formation. The disulfide arrangements for the recombinant protein were found to be identical to those in the native protein isolated from bovine brain.(ABSTRACT TRUNCATED AT 250 WORDS)

3/3,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07875528 94013246 PMID: 8408430
Structural characterisation of native and recombinant forms of the neurotrophic cytokine MK.
Fabri L; Maruta H; Muramatsu H; Muramatsu T; Simpson R J; Burgess A W; Nice E C
Melbourne Tumour Biology Branch, Ludwig Institute for Cancer Research (Melbourne Branch), Royal Melbourne Hospital, Victoria, Australia. Journal of chromatography (NETHERLANDS) Aug 27 1993, 646 (1) p213-25 , ISSN 0021-9673 Journal Code: 0427043
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The retinoic acid (RA)-inducible midkine (MK) gene encodes a heparin-binding protein which can induce neurite outgrowth in cultured mammalian embryonic brain cells. This cytokine shares 65% amino acid sequence identity with another RA-inducible cytokine, %%%pleiotropin%%% (PTN). Both proteins contain 10 conserved cysteine residues, all of which appear to be disulphide linked. MK and PTN are also rich in lysine and arginine residues rendering them susceptible to proteolysis during purification, and making large-scale preparation of these molecules inherently difficult. Recombinant MK has been expressed as a %%%fusion%%% %%%protein%%% using a pGEX vector transfected into E. coli. To enable refolding of MK, the %%%fusion%%% %%%protein%%% was stored in solution at 4 degrees C for 14 days in the presence of dithiothreitol (DTT). Thrombin cleavage of the %%%fusion%%% %%%protein%%% , post storage, typically generated 5 mg of MK per litre of bacterial pellet. To establish the structural integrity of the recombinant product, we have analysed the refolding kinetics and compared the disulphide bond assignment of recombinant MK with that of native MK and native PTN. The synergistic use of micropreparative HPLC, to separate and recover in small eluant volumes enzymatically derived peptide fragments, with matrix assisted laser desorption mass spectrometry (MALD-MS) and N-terminal sequence analysis has allowed the unambiguous identification of the disulphide bonded fragments of native and recombinant MK. The disulphide bond assignment of MK is C12-C36, C20-C45, C27-C49, C59-C91 and C69-C101, and is equivalent to that of PTN.
? s angiogenic()fusion()protein?
12548 ANGIOGENIC
172696 FUSION
2892396 PROTEIN?
S4 0 ANGIOGENIC()FUSION()PROTEIN?
? ds

Set Items Description
S1 61 PLEIOTROPIN OR HBNF

S2 2 S1 AND FUSION()PROTEIN?
S3 2 RD (unique items)
S4 0 ANGIOGENIC()FUSION()PROTEIN?
? s s1 and vegf
61 S1
13778 VEGF
S5 1 S1 AND VEGF
? t s5/3,ab/

5/3,AB/1 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
(c) 2002 BIOSIS. All rts. reserv.

12378891 BIOSIS NO.: 200000132393
Choroid plexus recovery after transient forebrain ischemia: Role of growth factors and other repair mechanisms.
AUTHOR: Johanson Conrad E(a); Palm Donald E; Primiano Michael J; McMillan Paul N; Chan Percy; Knuckey Neville W; Stopa Edward G
AUTHOR ADDRESS: (a)Department of Clinical Neurosciences, Rhode Island Hospital, 593 Eddy Street, Providence, RI, 02903**USA
JOURNAL: Cellular and Molecular Neurobiology. 20 (2):p197-216 April, 2000 ISSN: 0272-4340
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: 1. Transient forebrain ischemia in adult rats, induced by 10 min of bilateral carotid occlusion and an arterial hypotension of 40 mmHg, caused substantial damage not only to CA-1 neurons in hippocampus but also to epithelial cells in lateral ventricle choroid plexus. 2. When transient forebrain ischemia was followed by reperfusion (recovery) intervals of 0 to 12 hr, there was moderate to severe damage to many frond regions of the choroidal epithelium. In some areas, epithelial debris was sloughed into cerebrospinal fluid (CSF). Although some epithelial cells were disrupted and necrotic, their neighbors exhibited normal morphology. This patchy response to ischemia was probably due to regional differences in reperfusion or cellular metabolism. 3. Between 12 and 24 hr postischemia, there was marked restoration of the Na+, K+, water content, and ultrastructure of the choroid plexus epithelium. Since there was no microscopical evidence for mitosis, we postulate that healthy epithelial cells either were compressed together on the villus or migrated from the choroid plexus stalk to more distal regions, in order to "fill in gaps" along the basal lamina caused by necrotic epithelial cell disintegration. 4. Epithelial cells of mammalian choroid plexus synthesize and secrete many growth factors and other peptides that are of trophic benefit following injury to regions of the cerebroventricular system. For example, several growth factors are upregulated in choroid plexus after ischemic and traumatic insults to the central nervous system. 5. The presence of numerous types of growth factor receptors in choroid plexus allows growth factor mediation of recovery processes by autocrine and paracrine mechanisms. 6. The capability of choroid plexus after acute ischemia to recover its barrier and CSF formation functions is an important factor in stabilizing brain fluid balance. 7. Moreover, growth factors secreted by choroid plexus into CSF are distributed by diffusion and convection into brain tissue near the ventricular system, e.g., hippocampus. By this endocrine-like mechanism, growth factors are conveyed throughout the choroid plexus-CSF-brain nexus and can consequently promote repair of ischemia-damaged tissue in the ventricular wall and underlying brain.

2000
? t s5/kwic/

5/KWIC/1 (Item 1 from file: 5)
DIALOG(R)File 5:(c) 2002 BIOSIS. All rts. reserv.

DESCRIPTORS:
CHEMICALS & BIOCHEMICALS: ...%%%HBNF%%%--...

...%%%VEGF%%%--
? s multifunctional and angiogenic
11393 MULTIFUNCTIONAL
12548 ANGIOGENIC
S6 99 MULTIFUNCTIONAL AND ANGIOGENIC
? s fusion
S7 172696 FUSION
? s s6 aand s7
>>>Term "AAND" in invalid position
? s s6 and s7
99 S6
172696 S7
S8 1 S6 AND S7
? t s8/3,ab/

8/3,AB/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11301358 21341700 PMID: 11448908
Induction of interleukin-8 by Epstein-Barr virus latent membrane protein-1 and its correlation to angiogenesis in nasopharyngeal carcinoma. Yoshizaki T; Horikawa T; Qing-Chun R; Wakisaka N; Takeshita H; Sheen T S; Lee S Y; Sato H; Furukawa M
Department of Otolaryngology, School of Medicine, Kanazawa University, Kanazawa 920-8641, Japan. tomoy@med.kanazawa-u.ac.jp
Clinical cancer research : an official journal of the American Association for Cancer Research (United States) Jul 2001, 7 (7) p1946-51, ISSN 1078-0432 Journal Code: 9502500
Document type: Journal Article
Languages: ENGLISH

Main Citation Owner: NLM
Record type: Completed
PURPOSE: The EBV latent membrane protein-1 (LMP-1) is a %%%multifunctional%%% protein. Recently, the contribution of LMP-1 to the metastasis of nasopharyngeal carcinoma (NPC) has been suggested. Angiogenesis is a key step for metastasis. Thus, the association of LMP-1 to neovascularization of NPC was examined in this study. EXPERIMENTAL DESIGN: The association of LMP-1 to angiogenesis in 39 patients with NPC was evaluated by immunohistochemical study, and then induction of %%%angiogenic%%% factors by LMP-1 was examined by ELISA and luciferase reporter assay. RESULTS: In an immunohistochemical study, the expression of LMP-1 was significantly correlated to microvessel counts (P = 0.0003), suggesting that LMP-1 may induce some %%%angiogenic%%% factors. Therefore, we studied the relationship between LMP-1 expression and interleukin-8 (IL-8), vascular endothelial growth factor (VEGF), and basic fibroblast growth factor (bFGF) expression by immunohistochemical analysis. IL-8, VEGF, and bFGF expression were correlated to microvessel counts, but only IL-8 expression was significantly correlated to LMP-1 expression (P < 0.0001). Transfection with LMP-1 expression plasmid induced IL-8 protein expression in C33A cells. The expression of LMP-1 transactivated IL-8 promoter, as demonstrated by IL-8 promoter luciferase reporter assay. Mutation of the nuclear factor kappaB responsive element in the IL-8 promoter region completely abolished transactivation by LMP-1, whereas mutation of the activator protein responsive element did not affect promoter activity. CONCLUSION: These results suggested that LMP-1 induces expression of IL-8 through the nuclear factor kappaB binding site, which may contribute in part to angiogenesis in NPC.

```
? s bifunctional and fusion
       10637  BIFUNCTIONAL
      172696  FUSION
    S9    615  BIFUNCTIONAL AND FUSION
? s s9 and angiogenic
         615  S9
       12548  ANGIOGENIC
   S10     0  S9 AND ANGIOGENIC
? s s9 and vegf
         615  S9
       13778  VEGF
   S11     0  S9 AND VEGF
? ds

Set    Items  Description
S1        61  PLEIOTROPIN OR HBNF
S2         2  S1 AND FUSION()PROTEIN?
S3         2  RD (unique items)
S4         0  ANGIOGENIC()FUSION()PROTEIN?
S5         1  S1 AND VEGF
S6        99  MULTIFUNCTIONAL AND ANGIOGENIC
S7    172696  FUSION
S8         1  S6 AND S7
S9       615  BIFUNCTIONAL AND FUSION
S10        0  S9 AND ANGIOGENIC
S11        0  S9 AND VEGF
? logoff
     20oct02 09:05:49 User217743 Session D572.4
        $3.92   1.226 DialUnits File155
          $0.63  3 Type(s) in Format  4 (UDF)
        $0.63  3 Types
    $4.55  Estimated cost File155
        $4.35   0.778 DialUnits File5
          $0.16  1 Type(s) in Format 95 (KWIC)
          $1.75  1 Type(s) in Format  4 (UDF)
        $1.91  2 Types
    $6.26  Estimated cost File5
        OneSearch, 2 files,  2.004 DialUnits FileOS
    $0.86  TELNET
   $11.67  Estimated cost this search
   $13.03  Estimated total session cost   2.898 DialUnits
Logoff: level 02.09.15 D  09:05:49
```

```
      (c) 2002 The Dialog Corporation

    Set  Items  Description
    ---  -----  -----------
? set hi %%%;set hi %%%
HILIGHT set on as '%%%'%%%
%%%HILIGHT set on as '%%%'
? b 411
     20oct02 09:01:13 User217743 Session D572.2
        $0.00    0.070 DialUnits File410
    $0.00  Estimated cost File410
    $0.01  TELNET
    $0.01  Estimated cost this search
    $0.01  Estimated total session cost   0.244 DialUnits
File 411:DIALINDEX(R)

DIALINDEX(R)
  (c) 2002 The Dialog Corporation plc

*** DIALINDEX search results display in an abbreviated
*** *** format unless you enter the SET DETAIL ON
command. *** ? set files biochem
>>>       162 is unauthorized
>>>1 of the specified files is not available
  You have 22 files in your file list.
  (To see banners, use SHOW FILES command)
? s angiogenic()fusion()protein?

Your SELECT statement is:
  s angiogenic()fusion()protein?

       Items   File
       -----   ----

  No files have one or more items; file list includes 22
files.
? s pleiotropin or hbnf

Your SELECT statement is:
  s pleiotropin or hbnf

       Items   File
       -----   ----
         37    5: Biosis Previews(R)_1969-2002/Oct W2
       20    34: SciSearch(R) Cited Ref
Sci_1990-2002/Oct W3              3    50: CAB
Abstracts_1972-2002/Sep
          1    65: Inside Conferences_1993-2002/Oct W2
          5    71: ELSEVIER BIOBASE_1994-2002/Oct
W2
         20    73: EMBASE_1974-2002/Oct W2
          2    98: General Sci
Abs/Full-Text_1984-2002/Sep            27   144:
Pascal_1973-2002/Oct W2
         24   155: MEDLINE(R)_1966-2002/Oct W2
          1   156: ToxFile_1965-2002/Oct W3
          1   305: Analytical Abstracts_1980-2002/Oct
W1           21   399: CA
SEARCH(R)_1967-2002/UD=13716
          1   434: SciSearch(R) Cited Ref
Sci_1974-1989/Dec
  13 files have one or more items; file list includes 22
files.
? rf
Your last SELECT statement was:
  S PLEIOTROPIN OR HBNF

Ref      Items   File
---      -----   ----
N1          37    5: Biosis Previews(R)_1969-2002/Oct W2
N2          27   144: Pascal_1973-2002/Oct W2
N3          24   155: MEDLINE(R)_1966-2002/Oct W2
N4          21   399: CA
SEARCH(R)_1967-2002/UD=13716
N5          20    34: SciSearch(R) Cited Ref
Sci_1990-2002/Oct W3 N6          20    73:
EMBASE_1974-2002/Oct W2
N7           5    71: ELSEVIER BIOBASE_1994-2002/Oct
W2
N8           3    50: CAB Abstracts_1972-2002/Sep
N9           2    98: General Sci
Abs/Full-Text_1984-2002/Sep N10          1    65: Inside
Conferences_1993-2002/Oct W2    13 files have one or
more items; file list includes 22 files.
      - Enter P or PAGE for more -
? b 155, 5
     20oct02 09:02:12 User217743 Session D572.3
        $1.14    0.650 DialUnits File411
    $1.14  Estimated cost File411
    $0.21  TELNET
    $1.35  Estimated cost this search
    $1.36  Estimated total session cost   0.895 DialUnits
SYSTEM:OS  - DIALOG OneSearch
  File 155:MEDLINE(R)  1966-2002/Oct W2
*File 155: Alert feature enhanced for multiple files,
duplicates removal, customized scheduling. See HELP
ALERT.
  File   5:Biosis Previews(R)  1969-2002/Oct W2
       (c) 2002 BIOSIS
*File   5: Alert feature enhanced for multiple files,
duplicates removal, customized scheduling. See HELP
ALERT.

    Set  Items  Description
    ---  -----  -----------
? s pleiotropin or hbnf
          28  PLEIOTROPIN
          35  HBNF
    S1    61  PLEIOTROPIN OR HBNF
? s s1 and fusion()protein?
          61  S1
      172696  FUSION
     2892396  PROTEIN?
       69000  FUSION(W)PROTEIN?
    S2     2  S1 AND FUSION()PROTEIN?
? rd
```

...completed examining records
S3 2 RD (unique items)
? t s3/3,ab/1,2

3/3,AB/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08094895 94220851 PMID: 8167469
Refolding and characterization of human recombinant heparin-binding neurite-promoting factor.
Seddon A P; Hulmes J D; Decker M M; Kovesdi I; Fairhurst J L; Backer J; Dougher-Vermazen M; Bohlen P
Department of Protein Chemistry, American Cyanamid Company, Lederle Laboratories, Pearl River, New York 10965.
Protein expression and purification (UNITED STATES) Feb 1994, 5 (1) p14-21, ISSN 1046-5928 Journal Code: 9101496
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Heparin-binding neurite-promoting factor (%%%HBNF%%%) is a highly basic, cysteine-rich 136-residue protein, and a member of a new class of heparin-binding proteins. It exhibits a neurite-outgrowth promoting activity and its expression is both temporally and spacially regulated during fetal and postnatal development. A high interspecies sequence conservation suggests important, presently unknown, biological functions. %%%HBNF%%% is structurally and most likely functionally related to the product of a developmentally regulated gene, MK (midkine). To elucidate biological roles of these proteins, recombinant forms of the proteins were produced. Expression of human recombinant %%%HBNF%%% and MK in Escherichia coli lead to the formation of insoluble aggregated protein that accounted for about 25% of the total cellular protein. Homogeneous, monomeric forms of each protein were recovered from inclusion bodies by reduction with dithiothreitol and solubilization in 8 M urea. Refolding of the reduced and denatured protein occurred upon dialysis at pH 7.4. Human recombinant (hr) %%%HBNF%%% and hrMK prepared in this manner were further purified by heparin affinity chromatography. Chromatographic evidence demonstrates that refolding and concomitant disulfide bond formation in hrHBNF proceeds in high yield with minimal formation of stable nonnative disulfides. Studies on the redox status of the 10 cysteine residues of bovine brain %%%HBNF%%% and the refolded recombinant protein indicate that all cysteines are engaged in disulfide bond formation. The disulfide arrangements for the recombinant protein were found to be identical to those in the native protein isolated from bovine brain.(ABSTRACT TRUNCATED AT 250 WORDS)

3/3,AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07875528 94013246 PMID: 8408430
Structural characterisation of native and recombinant forms of the neurotrophic cytokine MK.
Fabri L; Maruta H; Muramatsu H; Muramatsu T; Simpson R J; Burgess A W; Nice E C
Melbourne Tumour Biology Branch, Ludwig Institute for Cancer Research (Melbourne Branch), Royal Melbourne Hospital, Victoria, Australia. Journal of chromatography (NETHERLANDS) Aug 27 1993, 646 (1) p213-25 , ISSN 0021-9673 Journal Code: 0427043
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The retinoic acid (RA)-inducible midkine (MK) gene encodes a heparin-binding protein which can induce neurite outgrowth in cultured mammalian embryonic brain cells. This cytokine shares 65% amino acid sequence identity with another RA-inducible cytokine, %%%pleiotropin%%% (PTN). Both proteins contain 10 conserved cysteine residues, all of which appear to be disulphide linked. MK and PTN are also rich in lysine and arginine residues rendering them susceptible to proteolysis during purification, and making large-scale preparation of these molecules inherently difficult. Recombinant MK has been expressed as a %%%fusion%%% %%%protein%%% using a pGEX vector transfected into E. coli. To enable refolding of MK, the %%%fusion%%% %%%protein%%% was stored in solution at 4 degrees C for 14 days in the presence of dithiothreitol (DTT). Thrombin cleavage of the %%%fusion%%% %%%protein%%% , post storage, typically generated 5 mg of MK per litre of bacterial pellet. To establish the structural integrity of the recombinant product, we have analysed the refolding kinetics and compared the disulphide bond assignment of recombinant MK with that of native MK and native PTN. The synergistic use of micropreparative HPLC, to separate and recover in small eluant volumes enzymatically derived peptide fragments, with matrix assisted laser desorption mass spectrometry (MALD-MS) and N-terminal sequence analysis has allowed the unambiguous identification of the disulphide bonded fragments of native and recombinant MK. The disulphide bond assignment of MK is C12-C36, C20-C45, C27-C49, C59-C91 and C69-C101, and is equivalent to that of PTN.
? s angiogenic()fusion()protein?
12548 ANGIOGENIC
172696 FUSION
2892396 PROTEIN?
S4 0 ANGIOGENIC()FUSION()PROTEIN?
? ds

Set Items Description
S1 61 PLEIOTROPIN OR HBNF

S2 2 S1 AND FUSION()PROTEIN?
S3 2 RD (unique items)
S4 0 ANGIOGENIC()FUSION()PROTEIN?
? s s1 and vegf
61 S1
13778 VEGF
S5 1 S1 AND VEGF
? t s5/3,ab/

5/3,AB/1 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

12378891 BIOSIS NO.: 200000132393
Choroid plexus recovery after transient forebrain ischemia: Role of growth factors and other repair mechanisms.
AUTHOR: Johanson Conrad E(a); Palm Donald E; Primiano Michael J; McMillan Paul N; Chan Percy; Knuckey Neville W; Stopa Edward G
AUTHOR ADDRESS: (a)Department of Clinical Neurosciences, Rhode Island Hospital, 593 Eddy Street, Providence, RI, 02903**USA
JOURNAL: Cellular and Molecular Neurobiology. 20 (2):p197-216 April, 2000 ISSN: 0272-4340
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: 1. Transient forebrain ischemia in adult rats, induced by 10 min of bilateral carotid occlusion and an arterial hypotension of 40 mmHg, caused substantial damage not only to CA-1 neurons in hippocampus but also to epithelial cells in lateral ventricle choroid plexus. 2. When transient forebrain ischemia was followed by reperfusion (recovery) intervals of 0 to 12 hr, there was moderate to severe damage to many frond regions of the choroidal epithelium. In some areas, epithelial debris was sloughed into cerebrospinal fluid (CSF). Although some epithelial cells were disrupted and necrotic, their neighbors exhibited normal morphology. This patchy response to ischemia was probably due to regional differences in reperfusion or cellular metabolism. 3. Between 12 and 24 hr postischemia, there was marked restoration of the Na+, K+, water content, and ultrastructure of the choroid plexus epithelium. Since there was no microscopical evidence for mitosis, we postulate that healthy epithelial cells either were compressed together on the villus or migrated from the choroid plexus stalk to more distal regions, in order to "fill in gaps" along the basal lamina caused by necrotic epithelial cell disintegration. 4. Epithelial cells of mammalian choroid plexus synthesize and secrete many growth factors and other peptides that are of trophic benefit following injury to regions of the cerebroventricular system. For example, several growth factors are upregulated in choroid plexus after ischemic and traumatic insults to the central nervous system. 5. The presence of numerous types of growth factor receptors in choroid plexus allows growth factor mediation of recovery processes by autocrine and paracrine mechanisms. 6. The capability of choroid plexus after acute ischemia to recover its barrier and CSF formation functions is an important factor in stabilizing brain fluid balance. 7. Moreover, growth factors secreted by choroid plexus into CSF are distributed by diffusion and convection into brain tissue near the ventricular system, e.g., hippocampus. By this endocrine-like mechanism, growth factors are conveyed throughout the choroid plexus-CSF-brain nexus and can consequently promote repair of ischemia-damaged tissue in the ventricular wall and underlying brain.

2000
? t s5/kwic/

5/KWIC/1 (Item 1 from file: 5)
DIALOG(R)File 5:(c) 2002 BIOSIS. All rts. reserv.

DESCRIPTORS:
CHEMICALS & BIOCHEMICALS: ...%%%HBNF%%%--...

...%%%VEGF%%%--
? s multifunctional and angiogenic
11393 MULTIFUNCTIONAL
12548 ANGIOGENIC
S6 99 MULTIFUNCTIONAL AND ANGIOGENIC
? s fusion
S7 172696 FUSION
? s s6 aand s7
>>>Term "AAND" in invalid position
? s s6 and s7
99 S6
172696 S7
S8 1 S6 AND S7
? t s8/3,ab/

8/3,AB/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11301358 21341700 PMID: 11448908
Induction of interleukin-8 by Epstein-Barr virus latent membrane protein-1 and its correlation to angiogenesis in nasopharyngeal carcinoma.
Yoshizaki T; Horikawa T; Qing-Chun R; Wakisaka N; Takeshita H; Sheen T S; Lee S Y; Sato H; Furukawa M
Department of Otolaryngology, School of Medicine, Kanazawa University, Kanazawa 920-8641, Japan. tomoy@med.kanazawa-u.ac.jp
Clinical cancer research : an official journal of the American Association for Cancer Research (United States) Jul 2001, 7 (7) p1946-51, ISSN 1078-0432 Journal Code: 9502500
Document type: Journal Article
Languages: ENGLISH

Main Citation Owner: NLM
Record type: Completed
PURPOSE: The EBV latent membrane protein-1 (LMP-1) is a %%%multifunctional%%% protein. Recently, the contribution of LMP-1 to the metastasis of nasopharyngeal carcinoma (NPC) has been suggested. Angiogenesis is a key step for metastasis. Thus, the association of LMP-1 to neovascularization of NPC was examined in this study. EXPERIMENTAL DESIGN: The association of LMP-1 to angiogenesis in 39 patients with NPC was evaluated by immunohistochemical study, and then induction of %%%angiogenic%%% factors by LMP-1 was examined by ELISA and luciferase reporter assay. RESULTS: In an immunohistochemical study, the expression of LMP-1 was significantly correlated to microvessel counts (P = 0.0003), suggesting that LMP-1 may induce some %%%angiogenic%%% factors. Therefore, we studied the relationship between LMP-1 expression and interleukin-8 (IL-8), vascular endothelial growth factor (VEGF), and basic fibroblast growth factor (bFGF) expression by immunohistochemical analysis. IL-8, VEGF, and bFGF expression were correlated to microvessel counts, but only IL-8 expression was significantly correlated to LMP-1 expression (P < 0.0001). Transfection with LMP-1 expression plasmid induced IL-8 protein expression in C33A cells. The expression of LMP-1 transactivated IL-8 promoter, as demonstrated by IL-8 promoter luciferase reporter assay. Mutation of the nuclear factor kappaB responsive element in the IL-8 promoter region completely abolished transactivation by LMP-1, whereas mutation of the activator protein responsive element did not affect promoter activity. CONCLUSION: These results suggested that LMP-1 induces expression of IL-8 through the nuclear factor kappaB binding site, which may contribute in part to angiogenesis in NPC.

```
? s bifunctional and fusion
       10637  BIFUNCTIONAL
      172696  FUSION
    S9    615  BIFUNCTIONAL AND FUSION
? s s9 and angiogenic
         615  S9
       12548  ANGIOGENIC
   S10     0  S9 AND ANGIOGENIC
? s s9 and vegf
         615  S9
       13778  VEGF
   S11     0  S9 AND VEGF
? ds

Set    Items  Description
S1        61  PLEIOTROPIN OR HBNF
S2         2  S1 AND FUSION()PROTEIN?
S3         2  RD (unique items)
S4         0  ANGIOGENIC()FUSION()PROTEIN?
S5         1  S1 AND VEGF
S6        99  MULTIFUNCTIONAL AND ANGIOGENIC
S7    172696  FUSION
S8         1  S6 AND S7
S9       615  BIFUNCTIONAL AND FUSION
S10        0  S9 AND ANGIOGENIC
S11        0  S9 AND VEGF
? logoff
     20oct02 09:05:49 User217743 Session D572.4
        $3.92   1.226 DialUnits File155
          $0.63  3 Type(s) in Format  4 (UDF)
        $0.63  3 Types
    $4.55  Estimated cost File155
        $4.35   0.778 DialUnits File5
          $0.16  1 Type(s) in Format 95 (KWIC)
          $1.75  1 Type(s) in Format  4 (UDF)
        $1.91  2 Types
    $6.26  Estimated cost File5
        OneSearch, 2 files,  2.004 DialUnits FileOS
    $0.86  TELNET
   $11.67  Estimated cost this search
   $13.03  Estimated total session cost   2.898 DialUnits
Logoff: level 02.09.15 D  09:05:49
```

```
     20oct02 10:11:04 User217743 Session D573.1
        $0.00   0.160 DialUnits FileHomeBase
    $0.00  Estimated cost FileHomeBase
    $0.00  Estimated cost this search
    $0.00  Estimated total session cost   0.160 DialUnits
File 410:Chronolog(R) 1981-2002/Sep
     (c) 2002 The Dialog Corporation

    Set  Items  Description
    ---  -----  -----------
? set hi *;set hi *
HILIGHT set on as '*'*
*HILIGHT set on as '*'
? b 155
     20oct02 10:11:08 User217743 Session D573.2
        $0.00   0.071 DialUnits File410
    $0.00  Estimated cost File410
    $0.01  TELNET
    $0.01  Estimated cost this search
    $0.01  Estimated total session cost   0.231 DialUnits
File 155:MEDLINE(R) 1966-2002/Oct W2
*File 155: Alert feature enhanced for multiple files,
duplicates removal, customized scheduling. See HELP
ALERT.

    Set  Items  Description
    ---  -----  -----------
? e au=kovesdi

Ref  Items  Index-term
E1       5  AU=KOVES Z
E2       0 *AU=KOVESDI
E3       2  AU=KOVESDI DOROTTYA
E4     105  AU=KOVESDI I
E5       4  AU=KOVESDI IMRE
E6       2  AU=KOVESDI J
E7       2  AU=KOVESDI J M
E8       2  AU=KOVESDI R
E9       3  AU=KOVESDI S
E10      2  AU=KOVESDY C
E11      6  AU=KOVESDY L
E12      1  AU=KOVESDY P

      Enter P or PAGE for more
? s e4,e5
         105  AU=KOVESDI I
           4  AU=KOVESDI IMRE
    S1     109  E4,E5
? s s1 and heparin()binding()neurotrophic
         109  S1
       52172  HEPARIN
      597548  BINDING
        8612  NEUROTROPHIC
          14  HEPARIN(W)BINDING(W)NEUROTROPHIC
    S2       2  S1 AND
HEPARIN()BINDING()NEUROTROPHIC
? t s2/3,ab/1,2
```


DIALOG(R)File 155:MEDLINE(R)

06953067 91265111 PMID: 2049182

Isolation from bovine brain and structural characterization of HBNF, a *heparin*-*binding* *neurotrophic* factor.

Bohlen P; Muller T; Gautschi-Sova P; Albrecht U; Rasool C G; Decker M; Seddon A; Fafeur V; *Kovesdi I*; Kretschmer P

Medical Research Division, American Cyanamid Company, Pearl River, NY 10965.

Growth factors (Chur, Switzerland) (SWITZERLAND) 1991, 4 (2) p97-107 , ISSN 0897-7194 Journal Code: 9000468

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

A heparin-binding protein with neurotrophic activity for perinatal rat neurons, termed HBNF, was purified to homogeneity from bovine brain utilizing pH 4.5 extraction, ammonium sulfate precipitation, cation exchange and heparin-Sepharose affinity chromatographies, and reverse phase HPLC. In the presence of protease inhibitors during extraction, a protein with an apparent molecular weight of 18 kDa was obtained in a yield of approximately 0.5 mg/kg brain tissue. The amino acid sequence of the first 114 residues of HBNF was determined and found to highly homologous to the cDNA-derived amino acid sequence of human HBNF, a 136-residue protein. Bovine and human HBNFs have identical molecular weights as judged by SDS gel electrophoresis and very similar amino acid compositions. This and overall sequence conservation suggest that bovine HBNF is also a 136 amino acid protein with a calculated molecular weight of approximately 15.5 kDa. The apparent discrepancy between calculated and observed molecular weights of bovine HBNF (and of human HBNF of which the complete sequence is known) is most likely a result of the highly basic nature of HBNF. If protease inhibitors were omitted during tissue extraction, two additional proteins with lower apparent molecular weights and identical N-terminal sequences were isolated, with the smallest forms being the major product. Amino acid analysis showed that the smaller forms correspond to C-terminally truncated HBNFs with calculated molecular weights of 13.6 and 12.4 kDa, lacking approximately 14 and 22 residues. Comparison of the HBNF protein sequence with sequences stored in the Protein Identification Resource/Genbank databases reveals high homology to the translation product of the MK-1 gene, which is retinoic acid-inducible in embryonic carcinoma cells and developmentally expressed during gestation in mice.

DIALOG(R)File 155:MEDLINE(R)

06741638 91054508 PMID: 1700712
Heparin-*binding* *neurotrophic* factor (HBNF) and MK, members of a new family of homologous, developmentally regulated proteins. *Kovesdi I*; Fairhurst J L; Kretschmer P J; Bohlen P Medical Research Division, American Cyanamid Company, Pearl River, NY 10965.
Biochemical and biophysical research communications (UNITED STATES) Oct 30 1990, 172 (2) p850-4, ISSN 0006-291X Journal Code: 0372516 Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
A partial rat cDNA clone coding for a novel neurotrophic factor HBNF was isolated. Nucleotide sequence determination, in combination with the known N-terminal sequence of rat HBNF, allowed deduction of the amino acid sequence of the first 102 residues of mature rat HBNF. HBNF shares high structural homology (55%) with the MK protein (Tomomura et al., J. Biol. Chem. 265, 10765, 1990). Complete alignment of 9 cysteine residues suggests further that the two proteins have similar 3-dimensional structures. HBNF was reported to stimulate neurite outgrowth in neurons and to be expressed in a developmentally regulated manner in the rat brain. MK mRNA was found in retinoid acid-induced teratocarcinoma cells and during early development of the mouse embryo, but no biological activity for MK is yet known. These data suggest that HBNF and MK are members of a novel family of structurally and probably functionally related proteins.
? s heparin()binding()neurotrophic and angiogenic
52172 HEPARIN
597548 BINDING
8612 NEUROTROPHIC
14 HEPARIN(W)BINDING(W)NEUROTROPHIC
5884 ANGIOGENIC
S3 0 HEPARIN()BINDING()NEUROTROPHIC AND ANGIOGENIC ? logoff
20oct02 10:12:30 User217743 Session D573.3
$1.78 0.555 DialUnits File155
$0.42 2 Type(s) in Format 4 (UDF)
$0.42 2 Types
$2.20 Estimated cost File155
$0.43 TELNET
$2.63 Estimated cost this search
$2.64 Estimated total session cost 0.786 DialUnits
Logoff: level 02.09.15 D 10:12:30

Connecting via Winsock to Dialog

Logging in to Dialog

Trying 31060000009999...Open

DIALOG INFORMATION SERVICES
PLEASE LOGON:
****
ENTER PASSWORD:
****
Welcome to DIALOG

Dialog level 02.09.15D

Last logoff: 20oct02 10:12:30
Logon file405 20oct02 10:35:33
HILIGHT set on as '*'
KWIC is set to 50.
* *
SYSTEM:HOME
Cost is in DialUnits
Menu System II: D2 version 1.7.8 term=ASCII
*** DIALOG HOMEBASE(SM) Main Menu ***
Information:
1. Announcements (new files, reloads, etc.)
2. Database, Rates, & Command Descriptions
3. Help in Choosing Databases for Your Topic
4. Customer Services (telephone assistance, training, seminars, etc.) 5. Product Descriptions

Connections:
6. DIALOG(R) Document Delivery
7. Data Star(R)

(c) 2000 The Dialog Corporation plc All rights reserved.
/H = Help /L = Logoff /NOMENU = Command Mode

Enter an option number to view information or to connect to an online service. Enter a BEGIN command plus a file number to search a database (e.g., B1 for ERIC).
? b 410

20oct02 10:35:34 User217743 Session D574.1
$0.00 0.153 DialUnits FileHomeBase
$0.00 Estimated cost FileHomeBase
$0.00 Estimated cost this search
$0.00 Estimated total session cost 0.153 DialUnits
File 410:Chronolog(R) 1981-2002/Sep
(c) 2002 The Dialog Corporation

Set Items Description
--- ----- -----------
? set hi *;set hi *
HILIGHT set on as '*'*
HILIGHT set on as ''
? b 155
20oct02 10:35:37 User217743 Session D574.2
$0.00 0.072 DialUnits File410

$0.00 Estimated cost File410
$0.00 Estimated cost this search
$0.00 Estimated total session cost 0.225 DialUnits
File 155:MEDLINE(R) 1966-2002/Oct W2
*File 155: Alert feature enhanced for multiple files, duplicates removal, customized scheduling. See HELP ALERT.

Set Items Description
--- ----- -----------
? s pleiotrophin
S1 209 PLEIOTROPHIN
? s s1 and angiogen?
209 S1
15733 ANGIOGEN?
S2 49 S1 AND ANGIOGEN?
? s s2 and py=2002
49 S2
394000 PY=2002
S3 9 S2 AND PY=2002
? t s2/3,ab/all

2/3,AB/1
DIALOG(R)File 155:MEDLINE(R)

13728183 22191306 PMID: 12070152
Dominant negative effectors of heparin affin regulatory peptide (HARP) *angiogenic* and transforming activities.
Bernard-Pierrot Isabelle; Delbe Jean; Rouet Vincent; Vigny Marc; Kerros Marie-Emmanuelle; Caruelle Daniele; Raulais Daniel; Barritault Denis; Courty Jose; Milhiet Pierre Emmanuel
Laboratoire de recherche sur la Croissance Cellulaire, la Reparation et la Regeneration Tissulaires (CRRET), CNRS UPRES-A 7053, Universite Paris XII, Avenue du General de Gaulle, 94010 Creteil Cedex, France. Journal of biological chemistry (United States) Aug 30 2002, 277 (35) p32071-7, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Heparin affin regulatory peptide (HARP) is an heparin-binding growth factor, highly expressed in several primary human tumors and considered as a rate-limiting *angiogenic* factor in tumor growth, invasion, and metastasis. Implication of this protein in carcinogenesis is linked to its mitogenic, *angiogenic*, and transforming activities. Recently, we have demonstrated that the C-terminal residues 111-136 of HARP are required for its mitogenic and transforming activities (Bernard-Pierrot, I., Delbe, J., Caruelle, D., Barritault, D., Courty, J., and Milhiet, P. E. (2001) J. Biol. Chem. 276, 12228-12234). In this paper, HARP deleted of its last 26 amino acids was shown to act as a dominant negative effector for its mitogenic, *angiogenic* , transforming, and tumor-formation activities by heterodimerizing with the wild type protein. Similarly, the synthetic corresponding peptide P111-136 displayed in vitro inhibition of wild type HARP activities, but in this case, the inhibition was mainly explained by the competition of the peptide with HARP for the binding to the extracellular domain of the high affinity ALK receptor.

2/3,AB/2
DIALOG(R)File 155:MEDLINE(R)

13687448 21933896 PMID: 11936877
The *angiogenic* factor heparin affin regulatory peptide (HARP) induces proliferation of human peripheral blood mononuclear cells. Achour A; Laaroubi D; Caruelle D; Barritault D; Courty J Laboratoire de Recherche sur la Croissance Cellulaire, la Reparation et la Regeneration Tissulaires (CRRET), CNRS UPRES-A 7053, Universite Paris Val de Marne, Creteil, France.
Cellular and molecular biology (Noisy-le-Grand, France) (France) 2001, 47 Online Pub pOL73-7, ISSN 0145-5680 Journal Code: 9216789 Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Heparin affin regulatory peptide (HARP) also named *pleiotrophin* (PTN) is a polypeptide that belongs to a family of heparin-binding molecules. HARP displays mitogenic activity for a wide variety of cells, including fibroblast, endothelial and epithelial cells. This study reports, to our knowledge for the first time that HARP induced the stimulation of triated thymidine incorporation in quiescent human peripheral blood mononuclear cells in a dose-dependant manner, measured after 7 days of culture. Maximal stimulation was observed at picomolar concentration with ED50 corresponding to the half maximum effect at 50 pM. In contrast, midkine (MK), a related heparin-binding growth/differentiation factor, with more than 50% amino acid sequence homology with HARP was ineffective. These results suggest that HARP could be considered as a new cytokine involved inthe growth regulation of cell mediated immunity.

2/3,AB/3
DIALOG(R)File 155:MEDLINE(R)

13597466 22229362 PMID: 12122009
Midkine Binds to Anaplastic Lymphoma Kinase (ALK) and Acts as a Growth Factor for Different Cell Types.
Stoica Gerald E; Kuo Angera; Powers Ciaran; Bowden Emma T; Sale Elaine Buchert; Riegel Anna T; Wellstein Anton
Lombardi Cancer Center, Georgetown University,

Washington, D. C. 2007. Journal of biological chemistry (United States) Sep 27 2002, 277 (39) p35990-8, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: In Process
Midkine (MK) is a developmentally regulated, secreted growth factor homologous to *pleiotrophin* (PTN). To investigate the potential role of MK in tumor growth, we expressed MK in human SW-13 cells and studied receptor binding, signal transduction, and activity of MK. The MK protein stimulates soft agar colony formation in vitro and tumor growth of SW-13 cells in athymic nude mice, as well as proliferation of human endothelial cells from brain microvasculature and umbilical vein (HUVEC) in the low ng/ml range. MK binds to anaplastic lymphoma kinase (ALK), the receptor for PTN, with an apparent K(d) of 170 pm in intact cells, and this receptor binding of MK is competed by PTN with an apparent K(d) of approximately 20 pm. Monoclonal antibodies raised against the extracellular ligand-binding domain of ALK inhibit ALK receptor binding of MK as well as MK-stimulated colony formation of SW-13 cells. Furthermore, MK stimulates ALK phosphorylation in WI-38 human fibroblasts and activates PI3-kinase and MAP kinase signal transduction in WI-38, HUVEC, neuroblastoma (SH SY-5Y) and glioblastoma (U87MG) cells that express the ALK protein. We conclude that MK can act as a growth, survival, and *angiogenic* factor during tumorigenesis and signals through the ALK receptor.

2/3,AB/4
DIALOG(R)File 155:MEDLINE(R)

13563217 22158524 PMID: 12168844
Anti-proliferative and antitumoral activities of a functionalized dextran (CMDBJ) on the 1205 L-U human tumor melanoma cells.
Bastias Jorge; Wei Ming X; Huynh Remi; Chaubet Frederic; Jozefonvicz Jacqueline; Crepin Michel
Laboratoire d' Oncologie Cellulaire et Moleculaire, UFR Leonard de Vinci, Universite Paris XIII, Bobigny, France.
Anticancer research (Greece) May-Jun 2002, 22 (3) p1603-13, ISSN 0250-7005 Journal Code: 8102988
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Carboxy methyl dextran benzylamide jorge (CMDBJ) is a derivatized dextran prepared from native dextran after random carboxymethylation of hydroxyl groups on D-glucose units (CM) and consecutive conversion of some carboxylate groups to benzylamide structures (B). This polymer exhibits an inhibitory action upon the proliferation of 1205 L-U human melanoma cells. At low concentrations, this compound exerts a cytostatic effect whereas, at higher concentrations, a cytotoxicity appears within 24 hours of treatment. The 1205 L-U cell line forms subcutaneous *angiogenic* tumors in athymic mice and, after several weeks, spontaneously forms micrometastasis in the lungs. We demonstrated that the CMDBJ treatment of animals not only reduces the rapid growth of primary tumors but also induces tumor regression and tumor necrosis. Moreover, CMDBJ treatment blocks the appearance of lung metastasis. *Pleiotrophin* (PTN), heparin-binding *angiogenic* growth factor, is secreted by 1205 L-U cells and breast tumor MDA-MB 231 cells. CMDBJ, as an inhibitor of heparin-binding growth factor activities, suppresses the mitogenic activity of conditioned media from 1205 L-U and MDA-MB 231 on endothelial HUVEC cells. We conclude that CMDBJ can inhibit the in vitro cell proliferation of 1205 L-U cells and 1205 L-U tumor development in athymic mice and that PTN secreted by these cells could be involved in this inhibition.

2/3,AB/5
DIALOG(R)File 155:MEDLINE(R)

13499575 21909932 PMID: 11912283
Pleiotrophin (PTN) and midkine (MK) mRNA expression in eutopic and ectopic endometrium in advanced stage endometriosis.
Chung Hye Won; Wen Yan; Choi Eun A; Hao-Li; Moon Hye Sung; Yu Han-Ki; Polan Mary Lake
Department of Obstetrics and Gynecology, Ewha Womans University School of Medicine, Seoul, Korea. hyewon@mm.ewha.ac.kr
Molecular human reproduction (England) Apr 2002, 8 (4) p350-5, ISSN 1360-9947 Journal Code: 9513710
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Endometriosis is characterized by the ectopic implantation of endometrium on peritoneal surfaces. *Angiogenic* and growth factors may play a significant role in the pathogenesis of endometriosis. Midkine (MK) and *pleiotrophin* (PTN) are two related peptides associated with carcinogenesis and *angiogenesis*. To test the hypothesis that a higher expression of MK and PTN in ectopic and eutopic endometrium from women with endometriosis might favour increased *angiogenesis* and growth with subsequent ectopic implantation, we investigated PTN and MK expression by quantitative competitive PCR (QC-PCR) in endometrium from 30 women with severe, stages III and IV endometriosis and from 30 women without endometriosis. Total RNA was extracted and reverse transcribed into cDNA, and QC-PCR was performed to evaluate PTN and MK mRNA expression. Results were analysed by analysis of variance. Eutopic endometrium from endometriosis

patients showed increased expression of MK and PTN mRNA compared with endometrium from normal women in the luteal phase ($P < 0.05$). MK and PTN mRNA expression in ectopic endometrium was significantly lower than that in eutopic endometrium from women with and without endometriosis ($P < 0.05$). Our results suggest increased MK and PTN expression may be related to the initiation of ectopic endometrial implants and peritoneal invasion.

2/3,AB/6
DIALOG(R)File 155:MEDLINE(R)

13437862 22041449 PMID: 12046056
The prognostic molecular markers in hepatocellular carcinoma. Qin Lun-Xiu; Tang Zhao-You
Liver Cancer Institute and Zhongshan Hospital, Fudan university, 136 Yi Xue Yuan Road, Shanghai 200032, China.
World J Gastroenterol (China) Jun 2002, 8 (3) p385-92, ISSN 1007-9327 Journal Code: 100883448
Document type: Journal Article; Review; Review, Academic Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The prognosis of hepatocellular carcinoma (HCC) still remains dismal, although many advances in its clinical study have been made. It is important for tumor control to identify the factors that predispose patients to death. With new discoveries in cancer biology, the pathological and biological prognostic factors of HCC have been studied quite extensively. Analyzing molecular markers (biomarkers) with prognostic significance is a complementary method. A large number of molecular factors have been shown to associate with the invasiveness of HCC, and have potential prognostic significance. One important aspect is the analysis of molecular markers for the cellular malignancy phenotype. These include alterations in DNA ploidy, cellular proliferation markers (PCNA, Ki-67, Mcm2, MIB1, MIA, and CSE1L/CAS protein), nuclear morphology, the p53 gene and its related molecule MD M2, other cell cycle regulators (cyclin A, cyclin D, cyclin E, cdc2, p27, p73), oncogenes and their receptors (such as ras, c-myc, c-fms, HGF, c-met, and erb-B receptor family members), apoptosis related factors (Fas and FasL), as well as telomerase activity. Another important aspect is the analysis of molecular markers involved in the process of cancer invasion and metastasis. Adhesion molecules (E-cadherin, catenins, serum intercellular adhesion molecule-1, CD44 variants), proteinases involved in the degradation of extracellular matrix (MMP-2, MMP-9, uPA, uPAR, PAI), as well as other molecules have been regarded as biomarkers for the malignant phenotype of HCC, and are related to prognosis and therapeutic outcomes. Tumor *angiogenesis* is critical to both the growth and metastasis of cancers including HCC, and has drawn much attention in recent years. Many *angiogenesis*-related markers, such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), platelet-derived endothelial cell growth factor (PD-ECGF), thrombospondin (TSP), *angiogenin*, *pleiotrophin*, and endostatin (ES) levels, as well as intratumor microvessel density (MVD) have been evaluated and found to be of prognostic significance. Body fluid (particularly blood and urinary) testing for biomarkers is easily accessible and useful in clinical patients. The prognostic significance of circulating DNA in plasma or serum, and its genetic alterations in HCC are other important trends. More attention should be paid to these two areas in future. As the progress of the human genome project advances, so does a clearer understanding of tumor biology, and more and more new prognostic markers with high sensitivity and specificity will be found and used in clinical assays. However, the combination of some items, i.e., the pathological features and some biomarkers mentioned above, seems to be more practical for now.

2/3,AB/7
DIALOG(R)File 155:MEDLINE(R)

13360851 22052249 PMID: 12057902
An invasion-independent pathway of blood-borne metastasis: a new murine mammary tumor model.
Sugino Takashi; Kusakabe Takashi; Hoshi Nobuo; Yamaguchi Tomiko; Kawaguchi Takanori; Goodison Steve; Sekimata Masayuki; Homma Yoshimi; Suzuki Toshimitsu
Department of Pathology, School of Medicine, Fukushima Medical University, Fukushima City, Japan. sugino@fmu.ac.jp
American journal of pathology (United States) Jun 2002, 160 (6) p1973-80, ISSN 0002-9440 Journal Code: 0370502
Comment in Am J Pathol. 2002 Jun;160(6) 1937-9; Comment in PMID 12057896 Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
It is generally believed that active invasion by cancer cells is essential to the metastatic process. In this report, we describe a murine mammary tumor (MCH66) model of metastasis that does not require invasion into the vascular wall of both the primary tumor and the target organ, in this case, the lung. The process involves intravasation of tumor nests surrounded by sinusoidal blood vessels, followed by intravascular tumor growth in the lung, without penetration of the vascular wall during the process. Comparative studies using a nonmetastatic MCH66 clone (MCH66C8) and another highly invasive metastatic cell line (MCH416) suggested that high *angiogenic* activity and sinusoidal

remodeling of tumor blood vessels were prerequisites for MCH66 metastasis. Differential cDNA analysis identified several genes that were overexpressed by MCH66, including genes for the *angiogenesis* factor *pleiotrophin*, and extracellular matrix-associated molecules that may modulate the microenvironment toward neovascularization. Our analyses suggest that tumor *angiogenesis* plays a role in the induction of invasion-independent metastasis. This model should prove useful in screening and development of new therapeutic agents for cancer metastasis.

2/3,AB/8
DIALOG(R)File 155:MEDLINE(R)

13359678 21401672 PMID: 11510908
Immunolocalization of the *angiogenetic* factor *pleiotrophin* (PTN) in the growth plate of mice.
Petersen W; Rafii M
Department of Orthopaedics, Christian-Albrechts-University Kiel, Germany. wolfpetersen@iname.com
Archives of orthopaedic and trauma surgery (Germany) Jul 2001, 121 (7) p414-6, ISSN 0936-8051 Journal Code: 9011043
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The aim of this study was to find out whether and where the *angiogenic* agent *pleiotrophin* (PTN) occurs within the growth plate. We investigated paraffin-embedded tissue sections of ten male mice with an antibody directed against the recombinant PTN. Immunostaining for PTN was positive within the cytoplasm and the pericellular matrix of osteoblasts which lined the longitudinal mineralized septae of the epiphyseal plate. Within the zone of hypertrophic chondrocytes, immunolabelling for PTN was positive in the pericellular matrix of hypertrophic chondrocytes and within the opened lacunae of the apoptotic hypertrophic chondrocytes. The resting zone and the proliferation zone were PTN negative. The results of our study suggest that the known *angiogenetic* peptide PTN plays a role in the process of *angiogenesis* in the growth plate.

2/3,AB/9
DIALOG(R)File 155:MEDLINE(R)

13142210 21993861 PMID: 11999218
Expression of *pleiotrophin* in hepatic nonparenchymal cells and preneoplastic nodules in carbon tetrachloride-induced fibrotic rat liver. Kohashi T; Tateaki Y; Tateno C; Asahara T; Obara M; Yoshizato K
Department of Biological Science, Graduate School of Science, Hiroshima University, Higashihiroshima, Japan.
Growth factors (Chur, Switzerland) (Switzerland) Mar 2002, 20 (1) p53-60, ISSN 0897-7194 Journal Code: 9000468
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: In Process
Pleiotrophin (PTN) is a heparin-binding protein, which induces growth, *angiogenesis* , differentiation, and transformation of cells. The aim of this study was to examine the role of PTN in liver fibrogenesis. Rats were treated with carbon tetrachloride (CCl4) for 3-9 weeks to induce liver fibrosis. The sirius-red staining of these liver tissue sections clearly showed the development of fibrosis and glutathione S-transferase placental type-positive preneoplastic nodules emerged at 7 weeks of the treatment. PTN expression was investigated in fibrotic liver tissues at the mRNA level using a real-time reverse transcription polymerase chain reaction and at the protein level by immunohistochemistry. Quantity of PTN mRNA increased 5-fold in fibrotic liver tissues at 7 weeks of CCl4-treatment over the control values. Immunohistochemistry localized PTN protein on hepatic nonparenchymal cells, mostly stellate cells and some of Kupffer cells, and the preneoplastic nodules in fibrotic liver tissues. PTN mRNA expression is significantly upregulated in the CCl4-induced chronic rat fibrotic liver tissues. We suggest that PTN might be involved in fibrogenesis and preneoplastic changes of liver.

2/3,AB/10
DIALOG(R)File 155:MEDLINE(R)

13093418 21949730 PMID: 11953815
Serum levels of the *angiogenic* factor *pleiotrophin* in relation to disease stage in lung cancer patients.
Jager R; List B; Knabbe C; Souttou B; Raulais D; Zeiler T; Wellstein A; Aigner A; Neubauer A; Zugmaier G
Department Hematology/Oncology, Center of Internal Medicine, Philipps University of Marburg, Baldingerstrasse, 35033 Marburg, Germany. zugmaier@mailer.uni-marburg.de
British journal of cancer (Scotland) Mar 18 2002, 86 (6) p858-63, ISSN 0007-0920 Journal Code: 0370635
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Pleiotrophin is a heparin-binding growth factor involved in the differentiation and proliferation of neuronal tissue during embryogenesis, and also secreted by melanoma and breast carcinoma cells. *Pleiotrophin* exhibits mitogenic and *angiogenic* properties and has been shown to influence the vascular

supply, expansion and metastasis of tumour cells. Our aim was to study the serum and plasma concentrations of *pleiotrophin* and the classical *angiogenic* growth factor vascular endothelial growth factor. Using a specific ELISA-test we studied patients with small cell lung cancer (n=63), and patients with non-small cell lung cancer (n=22) in comparison to healthy control subjects (n=41). In most of the lung cancer patients (81%), we found serum levels of *pleiotrophin* above those of control subjects (P<0.001). Of the 63 small cell lung cancer patients in the study *pleiotrophin* serum levels were elevated in 55 cases (87%) and in 14 cases (63%) of the 22 non-small cell lung cancer patients. *Pleiotrophin* mean serum concentrations were 10.8-fold higher in the tumour patient group as compared to the control group (P<0.001). Furthermore, *pleiotrophin* serum levels correlated positively with the stage of disease and inversely with the response to therapy. Plasma vascular endothelial growth factor concentrations were elevated in only in 28.6% of small cell lung cancer and 45.5% of non-small cell lung cancer patients by an average of 2.3-fold. Quite strikingly, there was no apparent correlation between the plasma vascular endothelial growth factor concentration and the stage of disease. Our study suggests that *pleiotrophin* may be an early indicator of lung cancer and might be of use in monitoring the efficacy of therapy, which needs to be confirmed by larger studies. Copyright 2002 Cancer Research UK

2/3,AB/11
DIALOG(R)File 155:MEDLINE(R)

12870392 21654713 PMID: 11795867
Pleiotrophin : a cytokine with diverse functions and a novel signaling pathway.
Deuel Thomas F; Zhang Nan; Yeh Hsui-Jen; Silos-Santiago Inmaculada; Wang Zhao-Yi
Division of Growth Regulation, Harvard Medical School, Boston, Massachusetts 02215, USA. tduel@caregroup.harvard.edu
Archives of biochemistry and biophysics (United States) Jan 15 2002, 397 (2) p162-71, ISSN 0003-9861 Journal Code: 0372430 Document type: Journal Article; Review; Review, Tutorial Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Pleiotrophin (PTN the protein, Ptn the gene) is a 136 amino acid secreted heparin-binding cytokine that signals diverse functions, including lineage-specific differentiation of glial progenitor cells, neurite outgrowth, and *angiogenesis*. *Pleiotrophin* gene expression is found in cells in early differentiation during different development periods and upregulated in cells with an early differentiation phenotype in wound repair. The Ptn gene is a protooncogene. It is strongly expressed in different human tumor cells and expression of the Ptn gene in tumor cells in vivo accelerates growth and stimulates tumor *angiogenesis* . Separate independent domains have been identified in PTN to signal transformation and tumor *angiogenesis*. *Pleiotrophin* is the first ligand of any of the known transmembrane tyrosine phosphatases. *Pleiotrophin* inactivates the receptor protein tyrosine phosphatase (RPTP) beta/zeta. The interaction of PTN and RPTP beta/zeta increases steady-state tyrosine phosphorylation of beta-catenin. *Pleiotrophin* thus regulates both normal cell functions and different pathological conditions at many levels. It signals these functions through a transmembrane tyrosine phosphatase. (c)2002 Elsevier Science.

2/3,AB/12
DIALOG(R)File 155:MEDLINE(R)

11228996 21264421 PMID: 11278720
Identification of anaplastic lymphoma kinase as a receptor for the growth factor *pleiotrophin*.
Stoica G E; Kuo A; Aigner A; Sunitha I; Souttou B; Malerczyk C; Caughey D J; Wen D; Karavanov A; Riegel A T; Wellstein A
Lombardi Cancer Center, Georgetown University, Washington, DC 20007, USA. Journal of biological chemistry (United States) May 18 2001, 276 (20) p16772-9, ISSN 0021-9258 Journal Code: 2985121R
Contract/Grant No.: CA58185; CA; NCI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Pleiotrophin (PTN) is a secreted growth factor that induces neurite outgrowth and is mitogenic for fibroblasts, epithelial, and endothelial cells. During tumor growth PTN can serve as an *angiogenic* factor and drive tumor invasion and metastasis. To identify a receptor for PTN, we panned a phage display human cDNA library against immobilized PTN protein as a bait. From this we isolated a phage insert that was homologous to an amino acid sequence stretch in the extracellular domain (ECD) of the orphan receptor tyrosine kinase anaplastic lymphoma kinase (ALK). In parallel with PTN, ALK is highly expressed during perinatal development of the nervous system and down-modulated in the adult. Here we show in cell-free assays as well as in radioligand receptor binding studies in intact cells that PTN binds to the ALK ECD with an apparent Kd of 32 +/- 9 pm. This receptor binding is inhibited by an excess of PTN, by the ALK ECD, and by anti-PTN and anti-ECD antibodies. PTN added to ALK-expressing cells induces phosphorylation of both ALK and of the downstream effector molecules IRS-1, Shc, phospholipase C-gamma, and phosphatidylinositol 3-kinase. Furthermore, the growth stimulatory effect of PTN on different cell lines in culture coincides with the endogenous expression

of ALK mRNA, and the effect of PTN is enhanced by ALK overexpression. From this we conclude that ALK is a receptor that transduces PTN-mediated signals and propose that the PTN-ALK axis can play a significant role during development and during disease processes.

2/3,AB/13
DIALOG(R)File 155:MEDLINE(R)

11179097 21192279 PMID: 11150308
The lysine-rich C-terminal tail of heparin affin regulatory peptide is required for mitogenic and tumor formation activities.
Bernard-Pierrot I; Delbe J; Caruelle D; Barritault D; Courty J; Milhiet P E
Laboratoire de Recherche sur la Croissance Cellulaire, la Reparation et la Regeneration Tissulaires, CNRS UPRES-A 7053, Universite Paris XII, Avenue du General de Gaulle, 94010 Creteil Cedex, France.
Journal of biological chemistry (United States) Apr 13 2001, 276 (15) p12228-34, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Heparin affin regulatory peptide (HARP) is a 18-kDa heparin-binding polypeptide that is highly expressed in developing tissues and in several primary human tumors. It seems to play a key role in cellular growth and differentiation. In vitro, HARP displays mitogenic, *angiogenic*, and neurite outgrowth activities. It is a secreted protein that is organized in two beta-sheet domains, each domain containing a cluster of basic residues. To assess determinants involved in the biological activities of HARP, C-terminally truncated proteins were produced in Chinese hamster ovary-K1 cells and tested for their mitogenic, tumor formation in nude mice and neurite outgrowth activities. Our data clearly indicate that the residues 111-136 of the lysine-rich C-terminal domain are involved in the mitogenic and tumor formation activities of HARP. Correlatively, no signal transduction was detected using the corresponding mutant, suggesting the absence of HARP binding to its high affinity receptor. However, this C-terminal domain of HARP is not involved in the neurite outgrowth activity. We also demonstrate that HARP signal peptide cleavage could led to two maturated forms that are both but differentially mitogenic.

2/3,AB/14
DIALOG(R)File 155:MEDLINE(R)

11165092 21182584 PMID: 11288955
Circulating *angiogenesis* regulators in cancer patients.
Kuroi K; Toi M
Department of Surgery, Tokyo Metropolitan Komagome Hospital, Japan. kurochan@dd.iij4u.or.jp
International journal of biological markers (Italy) Jan-Mar 2001, 16 (1) p5-26, ISSN 0393-6155 Journal Code: 8712411
Document type: Journal Article; Review; Review, Academic Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
BACKGROUND/AIMS: To date, numerous studies have demonstrated that several *angiogenesis* regulators circulate in the blood and may function as endocrine factors in cancer patients. This review aims to give a comprehensive insight into the possible clinical value of circulating *angiogenesis* regulators, mainly basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), and hepatocyte growth factor (HGF), *angiogenin*, *pleiotrophin*, thrombospondin (TSP) and endostatin (ES) in cancer patients. METHODS: A computerized (MEDLINE) and a manual search based on the reference lists of the publications were performed to identify articles published on this topic. RESULTS: In a detailed literature search, approximately 100 publications were found up to the end of 1999. Circulating *angiogenic* factors such as bFGF, VEGF, HGF and *angiogenin* have been evaluated not only as diagnostic and/or prognostic factors but also as predictive factors in cancer patients. On the other hand, little is known about the clinical significance of negative regulators. Neither the source nor the mechanism of protein externalization has been clarified in detail. CONCLUSIONS: Although there are no known factors with established clinical utility, circulating *angiogenesis* regulators may be useful in several situations. They could be used to determine the risk of developing cancer, to screen for early detection, to distinguish benign from malignant disease, and to distinguish between different types of malignancies. In patients with established malignancies such factors might be used to determine prognosis, to predict the response to therapy, and to monitor the clinical course. Further investigations are warranted to assess the specific utility of each factor.

2/3,AB/15
DIALOG(R)File 155:MEDLINE(R)

11152215 21164736 PMID: 11264008
HARP induces *angiogenesis* in vivo and in vitro: implication of N or C terminal peptides.
Papadimitriou E; Polykratis A; Courty J; Koolwijk P; Heroult M; Katsoris P
Laboratory of Molecular Pharmacology, University of Patras, Patras, Greece.
Biochemical and biophysical research communications (United States) Mar 23 2001, 282 (1) p306-13, ISSN 0006-291X Journal Code: 0372516 Document type: Journal Article

Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
HARP (heparin affin regulatory peptide) is a growth factor displaying high affinity for heparin. In the present work, we studied the ability of human recombinant HARP as well as its two terminal peptides (HARP residues 1-21 and residues 121-139) to promote *angiogenesis*. HARP stimulates endothelial cell tube formation on matrigel, collagen and fibrin gels, stimulates endothelial cell migration and induces *angiogenesis* in the in vivo chicken embryo chorioallantoic membrane assay. The two HARP peptides seem to be involved in most of the *angiogenic* effects of HARP. They both stimulate in vivo *angiogenesis* and in vitro endothelial cell migration and tube formation on matrigel. We conclude that HARP has an *angiogenic* activity when applied exogenously in several in vitro and in vivo models of *angiogenesis* and its NH(2) and COOH termini seem to play an important role. Copyright 2001 Academic Press.

2/3,AB/16
DIALOG(R)File 155:MEDLINE(R)

11124153 21135909 PMID: 11241349
Pleiotrophin induces *angiogenesis* : involvement of the phosphoinositide-3 kinase but not the nitric oxide synthase pathways. Souttou B; Raulais D; Vigny M
INSERM Unite 440/Universite Paris 6, Paris, France.
Journal of cellular physiology (United States) Apr 2001, 187 (1) p59-64, ISSN 0021-9541 Journal Code: 0050222
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Pleiotrophin (PTN) is a developmentally regulated protein that has been shown to be involved in tumor growth and metastasis presumably by activating tumor *angiogenesis*. To clarify the potential *angiogenic* activity of PTN and to analyze the signaling pathways involved in this process, we used an in vitro model of Human Umbilical Vein Endothelial Cells (HUVEC). We show that PTN was mitogenic toward a variety of endothelial cells including HUVEC, stimulated HUVEC migration across a reconstituted basement membrane and induced the formation of capillary-like structures by HUVEC grown as 3D-cultures in Matrigel or collagen. The signaling pathways triggered following endothelial cell stimulation by PTN were studied by using pharmacological inhibitors of the Phosphoinositide-3 kinase (PI3K) and endothelial Nitric Oxide Synthase (eNOS), two enzymes that have been shown to be crucial in the *angiogenic* response to Vascular Endothelial Growth Factor (VEGF). Whereas wortmannin (a PI3K inhibitor) and L-NAME (an eNOS inhibitor) dramatically reduced HUVEC growth induced by VEGF, only the former inhibitor reduced the growth induced by PTN and to a lesser extent that stimulated by basic Fibroblast Growth Factor. Thus, our results indicate that PTN induces *angiogenesis* and utilizes PI3K- but not eNOS-dependent pathways for its *angiogenic* activity. Copyright 2001 Wiley-Liss, Inc.

2/3,AB/17
DIALOG(R)File 155:MEDLINE(R)

11100958 21140293 PMID: 11244508
The *angiogenic* factor midkine is aberrantly expressed in NF1-deficient Schwann cells and is a mitogen for neurofibroma-derived cells.
Mashour G A; Ratner N; Khan G A; Wang H L; Martuza R L; Kurtz A Vincent T Lombardi Cancer Center, Department of Neurosurgery, Georgetown University School of Medicine, 3970 Reservoir Road NW, Washington DC 20007, USA.
Oncogene (England) Jan 4 2001, 20 (1) p97-105, ISSN 0950-9232 Journal Code: 8711562
Contract/Grant No.: R01-NS28840; NS; NINDS; R29-NS37895; NS; NINDS Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Loss of the tumor suppressor gene NF1 in neurofibromatosis type 1 (NF1) contributes to the development of a variety of tumors, including malignant peripheral nerve sheath tumors (MPNST) and benign neurofibromas. Of the different cell types found in neurofibromas, Schwann cells usually provide between 40 and 80%, and are thought to be critical for tumor growth. Here we describe the identification of growth factors that are upregulated in NF1-/- mouse Schwann cells and are potential regulators of *angiogenesis* and cell growth. Basic fibroblast growth factor (FGF-2), platelet-derived growth factor (PDGF) and midkine (MK) were found to be induced by loss of neurofibromin and MK was further characterized. MK was induced in human neurofibromas, schwannomas, and various nervous system tumors associated with NF1 or NF2; midkine showed an expression pattern overlapping but distinct from its homolog *pleiotrophin* (PTN). Immunohistochemistry revealed expression of MK in S-100 positive Schwann cells of dermal and plexiform neurofibromas, and in endothelial cells of tumor blood vessels, but not in normal blood vessels. Furthermore, MK demonstrated potent mitogenic activity for human systemic and brain endothelial cells in vitro and stimulated proliferation and soft agar colony formation of human MPNST derived S100 positive cells and fibroblastoid cells derived from an NF1 neurofibroma. The data support a possible central role for MK as a mediator of *angiogenesis* and neurofibroma growth in NF1. Oncogene (2001) 20, 97 - 105.

2/3,AB/18
DIALOG(R)File 155:MEDLINE(R)

10894843 20468874 PMID: 11016659
Pleiotrophin can be rate-limiting for pancreatic cancer cell growth.
Weber D; Klomp H J; Czubayko F; Wellstein A; Juhl H
Lombardi Cancer Center, Georgetown University Medical Center, Washington, DC, 20007, USA.
Cancer research (UNITED STATES) Sep 15 2000, 60 (18) p5284-8, ISSN 0008-5472 Journal Code: 2984705R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Pancreatic cancer is one of the most aggressive malignant tumors, with an overall survival rate of 2%. The identification of growth factors that contribute to the malignant phenotype can help to identify new targets for therapy. In this study, we analyzed the growth factor *pleiotrophin* (PTN) that was originally described as a developmentally regulated cytokine during early embryogenesis. More recently, PTN was found to be overexpressed in a variety of neuroectodermal tumors and described as an essential *angiogenic* growth factor in choriocarcinoma and melanoma, promoting metastatic growth. Recently, we discovered high expression levels of PTN in patients with gastrointestinal malignancies, particularly in those patients with pancreatic cancer. However, it is not known whether PTN is a contributor to the growth of pancreatic cancer or is only a bystander. We used ribozymes to deplete PTN mRNA from Colo357 pancreatic cancer cells and studied the resulting phenotype. The reduction of PTN resulted in a decrease in the proliferation rate, soft agar colony formation, and tumor growth in animals. Supplementation of cells with PTN partially reversed the ribozyme effect. The autocrine function of PTN was confirmed by using PTN-binding antibodies that inhibited the proliferation rate by 50% in Colo357 cells but also in a different pancreatic cancer cell line, Panc89. Our study identifies PTN as a new and essential growth factor for pancreatic cancer. Due to the restricted expression pattern of PTN in adults, PTN is suggested as a target for pancreatic cancer therapy.

2/3,AB/19
DIALOG(R)File 155:MEDLINE(R)

10791995 20337225 PMID: 10879061
[Recent progress of midkine research on cancer]
Kadomatsu K
Department of Biochemistry, Nagoya University School of Medicine. Nippon rinsho. Japanese journal of clinical medicine (JAPAN) Jun 2000, 58 (6) p1337-47, ISSN 0047-1852 Journal Code: 0420546 Document type: Journal Article; Review; Review, Tutorial ; English Abstract
Languages: JAPANESE
Main Citation Owner: NLM
Record type: Completed
Midkine is a heparin-binding growth factor, implicated in various biological phenomena such as neuronal survival and differentiation, tissue remodeling and carcinogenesis. Together with *pleiotrophin*, midkine constitutes a family that is distinct from other heparin-binding growth factors. In this review, I will briefly describe biochemical and biological characteristics of midkine and then focus on its biological significance in cancer. The most intriguing feature of midkine in cancer is its augmented expression in advanced tumors at very high frequency in non-tissue specific manner. In addition, its high expression is also detected in precancerous lesions. Midkine exerts carcinogenesis-related activities, including transforming, anti-apoptotic, *angiogenic* and fibrinolytic ones. These data provide a possibility of clinical application of midkine. Serum midkine level can be a useful tumor marker. Gene therapy using its promoter region and therapeutic strategy choosing midkine as a molecular target are worth challenging.

2/3,AB/20
DIALOG(R)File 155:MEDLINE(R)

10737805 20278617 PMID: 10818676
New paradigms for the treatment of cancer: the role of anti- *angiogenesis* agents.
Cherrington J M; Strawn L M; Shawver L K
SUGEN, Inc., South San Francisco, CA 94080, USA.
Advances in cancer research (UNITED STATES) 2000, 79 p1-38, ISSN 0065-230X Journal Code: 0370416
Document type: Journal Article; Review; Review, Academic Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Angiogenesis , the sprouting of new blood vessels, plays a role in diverse disease states including cancer, diabetic retinopathy, age-related macular degeneration, rheumatoid arthritis, psoriasis, atherosclerosis, and restenosis. With regard to cancer, the clinical association of tumor vascularity with tumor aggressiveness has been clearly demonstrated in numerous tumor types. The observation of increased microvessel density in tumors not only serves as an independent prognostic indicator, but also suggests that anti-*angiogenic* therapy may be an important component of treatment regimens for cancer patients. The complexity of the *angiogenic* process, which involves both positive and negative regulators, provides a number of targets for therapy. Many positive regulators, including growth factor receptors, matrix

metalloproteinases, and integrins, have been correlated with increased vascularity of tumors and poor prognosis for patient survival. Thus, these serve as ideal targets for anti-*angiogenesis* therapy. Many inhibitors of these targets are currently undergoing clinical evaluation as potential anti-cancer agents. In this article, we discuss the role of positive regulators in *angiogenesis* and tumor growth and describe the anti-*angiogenic* agents under development.

2/3,AB/21
DIALOG(R)File 155:MEDLINE(R)

10547964 20086641 PMID: 10618596
Chemical synthesis of proteins in solution.
Sakakibara S
Peptide Institute, Inc., Protein Research Foundation, Minoh-shi, Osaka 565-8686, Japan.
Biopolymers (UNITED STATES) 1999, 51 (4) p279-96, ISSN 0006-3525 Journal Code: 0372525
Document type: Journal Article; Review; Review, Tutorial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Development of a novel strategy suitable for the solution synthesis of proteins is described, wherein the entire molecule is assembled from fully protected segments in the size range of about 10 residues. Each segment is designed so as to have a common structure of Boc-peptide-OPac (Pac: phenacyl) and all of the side-chain functional groups are protected by Bzl-based groups. New types of solvent systems are described for dissolving fully protected segments in which the segment condensation reactions in solution can be carried out smoothly. After removal of the Boc or Pac group, the segments are coupled together to obtain the entire sequence using the 1-ethyl-3-(3'-dimethylaminopropyl)-carbodiimide/3, 4-dihydro-3-hy droxy-4-oxo-1,2,3-benzotriazine method. The side-chain protecting groups are then removed by HF and the liberated peptide is subjected to folding reactions to obtain the native conformation. Applying the strategy, the 123-residue human *angiogenin* , the 121-residue human midkine, the 136-residue human *pleiotrophin* , and the 238-residue Aequoria green fluorescent protein were synthesized successfully. Copyright 1999 John Wiley & Sons, Inc.

2/3,AB/22
DIALOG(R)File 155:MEDLINE(R)

10366207 99377411 PMID: 10448302
Melanoma cell-derived factor stimulation of fibroblast glycosaminoglycan synthesis--the role of platelet-derived growth factor.
Godden J L; Edward M; MacKie R M
Department of Dermatology, University of Glasgow, U.K.
European journal of cancer (Oxford, England : 1990) (ENGLAND) Mar 1999, 35 (3) p473-80, ISSN 0959-8049 Journal Code: 9005373 Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The hyaluronan-rich matrix surrounding many tumours may facilitate tumour growth, invasion and *angiogenesis* , with the majority of this hyaluronan apparently being synthesised by normal fibroblasts, stimulated to do so by tumour cell-derived factors. Melanoma cell-conditioned medium (CM) stimulates up to a 6-fold increase in fibroblast glycosaminoglycan (GAG) synthesis, with the active factors being present in tumour CM ultrafiltration fractions > 30 kDa and < 1 kDa. These fractions are poorly active individually, but when recombined, the activity is substantially greater than the additive effect. The objective of this study was to identify the factors present in the ultrafiltration fraction > 30 kDa that produce a greater than additive effect with the fraction < 1 kDa in stimulating the incorporation of 3H glucosamine into fibroblast GAGs. A number of factors including basic fibroblast growth factor (bFGF), interleukin (IL)-1 beta, *pleiotrophin*, platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-beta), tumour necrosis factor-alpha (TNF-alpha) and vascular endothelial growth factor (VEGF) failed to stimulate any significant increase in GAG synthesis, but when added to the < 1 kDa tumour CM fraction, both PDGF and to a lesser extent, bFGF, exhibited potent stimulating activities. Neutralising antibodies to PDGF and bFGF added to the melanoma CM decreased the fibroblast GAG-stimulating activity by 29% and 40%, respectively, in C8161 melanoma CM and by 47% and 45%, respectively, in Hs294T melanoma CM. The activities of PDGF-AA and PDGF-BB isoforms were indistinguishable, suggesting the PDGF-alpha receptor plays a role in the GAG-stimulatory response. Western analysis following treatment with PDGF, bFGF or melanoma CM revealed banding patterns for PDGF and melanoma CM that were similar. Immunoprecipitation of the PDGF-alpha receptor revealed it to be phosphorylated in fibroblasts treated with PDGF and melanoma CM, but not with control fibroblast CM. These studies suggest that PDGF plays an important role in the GAG-stimulating activity of the melanoma CM, but requires the presence of an as yet unidentified novel low molecular weight factor for full activity.

2/3,AB/23
DIALOG(R)File 155:MEDLINE(R)

10322842 99300992 PMID: 10372404
Implication of HARP in *angiogenesis* and possible

therapeutic role] Implication de l'HARP dans l'*angiogenese* et role therapeutique possible.
Delbe J; Katsoris P; Milhiet P E; Barritault D; Caruelle D; Courty J Laboratoire de Recherche sur la Croissance Cellulaire, la Reparation et la Regeneration Tissulaires (CRRET), UPRESA CNRS 7053, Universite Paris Val-de-Marne, Creteil, France.
Pathologie-biologie (FRANCE) Apr 1999, 47 (4) p352-7, ISSN 0369-8114 Journal Code: 0265365
Document type: Journal Article; Review; Review, Tutorial ; English Abstract
Languages: FRENCH
Main Citation Owner: NLM
Record type: Completed
HARP (heparin affin regulatory peptide), also called *pleiotrophin* (PTN), belongs to the heparin binding growth factors (HBGFs) family. Several new data suggest a role for HARP during the various stages of *angiogenesis* . In vivo, HARP is localised in endothelial cells of blood capillaries. In vitro, HARP displays mitogenic activity on endothelial cells, induces the formation of capillary-like structures in collagen gel, and degrades extracellular matrix via stimulation of plasminogen activator activity. HARP is also involved in neoangiogenesis during tumor progression. This review discusses the possible role of HARP in tumor *angiogenesis* and its therapeutic implications.

2/3,AB/24
DIALOG(R)File 155:MEDLINE(R)

10153828 99137383 PMID: 9973098
Involvement of heparin affin regulatory peptide in human prostate cancer. Vacherot F; Caruelle D; Chopin D; Gil-Diez S; Barritault D; Caruelle J P; Courty J
Laboratoire de Recherche sur la Croissance Cellulaire, la Reparation et la Regeneration Tissulaires, Universite Paris XII-Val de Marne, Creteil, France.
Prostate (UNITED STATES) Feb 1 1999, 38 (2) p126-36, ISSN 0270-4137 Journal Code: 8101368
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
BACKGROUND: Heparin affin regulatory peptide (HARP) composes, together with midkine (MK), a new family of heparin-binding growth/differentiation factors. Recently, HARP was incriminated in cancer progression, as an *angiogenic* factor and as a tumor growth factor. In this study, we analyzed the possible involvement of HARP in human prostate cancer (Pca). METHODS: The localization of HARP protein and its mRNAs in normal prostate (n = 5), benign prostate hyperplasia (BPH) (n = 7), and prostate cancer (Pca) (n = 9) was analyzed by immunohistochemistry and in situ hybridization. The mitogenic activity of this growth factor for prostate epithelial cells was determined with a thymidine incorporation assay. HARP cDNA was transfected into normal prostate epithelial (PNT-1A) cells, and their growth was evaluated by soft-agar growth assay. RESULTS: We found HARP protein associated with epithelial cells in PCa but not in normal prostate or BPH, while the corresponding mRNAs were located in the stromal compartment. Furthermore, HARP is mitogenic for PNT-1A, LNCaP, and DU-145 cells. Overexpression of the human HARP in PNT-1A transfected cells induced both anchorage-independent growth and growth at low serum concentrations. CONCLUSIONS: Our results suggest that HARP may act in a paracrine manner from mesenchymal to tumoral epithelial cells, and may play a role in the molecular mechanisms that regulate prostate tumor cell growth.

2/3,AB/25
DIALOG(R)File 155:MEDLINE(R)

10143711 99112759 PMID: 9915553
Pleiotrophin and midkine, a family of mitogenic and *angiogenic* heparin-binding growth and differentiation factors. Zhang N; Deuel T F
Division of Growth Regulation, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA 02215, USA.
Current opinion in hematology (UNITED STATES) Jan 1999, 6 (1) p44-50 , ISSN 1065-6251 Journal Code: 9430802
Document type: Journal Article; Review; Review, Tutorial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The heparin-binding polypeptide homologs *pleiotrophin* and midkine are the only known members of a family of secreted growth/differentiation cytokines. *Pleiotrophin* and midkine are both developmentally regulated and highly conserved among species. They signal a number of physiological functions involved with *angiogenesis*, neuorogenesis, cell migration, and mesoderm-epithelial interactions. Constitutive expression of *pleiotrophin* and midkine in responsive cells support their role as "tumor growth factors" and positive regulators of tumor *angiogenesis*. Widespread deregulation of *pleiotrophin* and midkine is found in many known human cancers or their derived cell lines, and the molecular targeting of *pleiotrophin* to block its signaling in tumor cells has limited tumor growth and metastasis in animal models. Elucidating the molecular mechanisms of *pleiotrophin* and midkine action in tumorgenesis and tumor *angiogenesis* may lead to the identification of novel targets for tumor therapy.

2/3,AB/26
DIALOG(R)File 155:MEDLINE(R)

10065126 99062598 PMID: 9846168
Upregulation of the *angiogenic* factor heparin affin

regulatory peptide by progesterone in rat uterus.
Milhiet P E; Vacherot F; Caruelle J P; Barritault D; Caruelle D; Courty J Laboratoire de recherche sur la Croissance Cellulaire, la Reparation et la Regeneration tissulaires (CRRET), CNRS UPRESA 7053, Universite Paris XII, Creteil, France.
Journal of endocrinology (ENGLAND) Sep 1998, 158 (3) p389-99, ISSN 0022-0795 Journal Code: 0375363
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Heparin affin regulatory peptide (HARP), also named pleiotropin, is a secreted polypeptide that belongs to a new family of heparin-binding growth/differentiation factors. In this study, we investigated the expression and distribution of HARP mRNA and protein in rat uterus. Semi-quantitative reverse transcriptase PCR experiments showed variations in HARP mRNA levels throughout the estrous cycle, with a maximum during diestrus, pointing to hormonal regulation of HARP mRNA expression. Uterine expression of HARP mRNA was studied in ovariectomized animals treated with 17 beta-estradiol, progesterone alone or progesterone and RU486. In these experiments, progesterone upregulated HARP mRNA expression. Induction was observed 6 h after progesterone injection and was inhibited by RU486 treatment. In contrast, after 17 beta-estradiol injection, a slight decrease in HARP mRNA expression was observed. In situ hybridization studies with digoxigenin-labeled DNA probe revealed that HARP mRNA was present in smooth muscle cells of both myometrium and blood vessels and also in endothelial cells from endometrium. Immunohistochemical studies showed that HARP expression was not limited to cells that expressed HARP mRNA, but also occurred in both the luminal and glandular epithelium even though its transcript was never detected. We conclude that HARP may mediate the effects of progesterone on the homeostasis and vascularization of uterine tissue.

2/3,AB/27
DIALOG(R)File 155:MEDLINE(R)

10044753 99030606 PMID: 9811837
Chemical synthesis of the precursor molecule of the Aequorea green fluorescent protein, subsequent folding, and development of fluorescence. Nishiuchi Y; Inui T; Nishio H; Bodi J; Kimura T; Tsuji F I; Sakakibara S Peptide Institute, Protein Research Foundation, Minoh-shi, Osaka 562, Japan.
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Nov 10 1998, 95 (23) p13549-54, ISSN 0027-8424 Journal Code: 7505876
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The present paper describes the total chemical synthesis of the precursor molecule of the Aequorea green fluorescent protein (GFP). The molecule is made up of 238 amino acid residues in a single polypeptide chain and is nonfluorescent. To carry out the synthesis, a procedure, first described in 1981 for the synthesis of complex peptides, was used. The procedure is based on performing segment condensation reactions in solution while providing maximum protection to the segment. The effectiveness of the procedure has been demonstrated by the synthesis of various biologically active peptides and small proteins, such as human *angiogenin*, a 123-residue protein analogue of ribonuclease A, human midkine, a 121-residue protein, and *pleiotrophin*, a 136-residue protein analogue of midkine. The GFP precursor molecule was synthesized from 26 fully protected segments in solution, and the final 238-residue peptide was treated with anhydrous hydrogen fluoride to obtain the precursor molecule of GFP containing two Cys(acetamidomethyl) residues. After removal of the acetamidomethyl groups, the product was dissolved in 0.1 M Tris. HCl buffer (pH 8.0) in the presence of DTT. After several hours at room temperature, the solution began to emit a green fluorescence (lambdamax = 509 nm) under near-UV light. Both fluorescence excitation and fluorescence emission spectra were measured and were found to have the same shape and maxima as those reported for native GFP. The present results demonstrate the utility of the segment condensation procedure in synthesizing large protein molecules such as GFP. The result also provides evidence that the formation of the chromophore in GFP is not dependent on any external cofactor.

2/3,AB/28
DIALOG(R)File 155:MEDLINE(R)

10008924 98447307 PMID: 9776412
Relationship between serum concentrations of the growth factor *pleiotrophin* and *pleiotrophin*-positive tumors. Souttou B; Juhl H; Hackenbruck J; Rockseisen M; Klomp H J; Raulais D; Vigny M; Wellstein A Lombardi Cancer Center and Department of Pharmacology, Georgetown University, Washington, DC 20007, USA.
Journal of the National Cancer Institute (UNITED STATES) Oct 7 1998, 90 (19) p1468-73, ISSN 0027-8874 Journal Code: 7503089 Contract/Grant No.: CA58185; CA; NCI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
BACKGROUND: Growth factors produced by tumor cells are essential for tumor expansion and may be

useful in monitoring tumor progression or therapeutic efficacy if the factors are released into the circulation. In this study, we measured serum levels of *pleiotrophin*, a secreted heparin-binding growth and *angiogenesis* factor, in mice bearing human tumor xenografts to determine whether these levels reflected overall tumor burden, and we examined the relationship between tumor expression of *pleiotrophin* and serum levels of this factor in patients with cancer. METHODS: *Pleiotrophin* in serum from mice and humans was measured by use of a highly sensitive enzyme-linked immunosorbent assay. For the clinical studies, serum specimens were obtained from 193 patients with various cancers of the gastrointestinal tract and from 28 healthy control subjects. In a subset of 64 cancer patients, serum levels of *pleiotrophin* were measured at the time of surgery, and tumor expression of this factor was detected immunohistochemically. All P values are two-sided. RESULTS: In mice, serum *pleiotrophin* levels were found to increase as a function of tumor size. In humans, elevated serum *pleiotrophin* levels were found in patients with pancreatic cancer (n = 41; P<.0001) and colon cancer (n = 65; P = .0079) but not in patients with stomach cancer (n = 87; P =.42). A statistically significant positive association was found between elevated levels of *pleiotrophin* in serum drawn at the time of surgery and expression of this factor by tumors (P<.0001). In both mice and humans, serum *pleiotrophin* levels dropped after successful tumor removal. CONCLUSIONS: Elevated serum *pleiotrophin* levels can indicate the presence of tumors expressing this factor. Monitoring serum levels of *pleiotrophin* may prove useful in determining the pharmacologic efficacy of cytotoxic or anti- *pleiotrophin* therapy.

2/3,AB/29
DIALOG(R)File 155:MEDLINE(R)

09803995 98237748 PMID: 9570800
Upregulation of *pleiotrophin* gene expression in developing microvasculature, macrophages, and astrocytes after acute ischemic brain injury.
Yeh H J; He Y Y; Xu J; Hsu C Y; Deuel T F
Department of Medicine, Division of Growth Regulation, Beth Israel Deaconess Medical Center, Boston, Massachusetts 02215, USA. Journal of neuroscience : the official journal of the Society for Neuroscience (UNITED STATES) May 15 1998, 18 (10) p3699-707, ISSN 0270-6474 Journal Code: 8102140
Contract/Grant No.: NS25545; NS; NINDS; NS28995; NS; NINDS; NS32636; NS; NINDS; +
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Pleiotrophin (PTN) is a heparin-binding, 18 kDa secretory protein that functions to induce mitogenesis, *angiogenesis*, differentiation, and transformation in vitro. PTN gene (Ptn) expression is highly regulated during development and is highest at sites in which mitogenesis, *angiogenesis* , and differentiation are active. In striking contrast, with the exception of the neuron, the Ptn gene is only minimally expressed in adults. We now demonstrate that Ptn gene expression is strikingly upregulated within 3 d in OX42-positive macrophages, astrocytes, and endothelial cells in areas of developing neovasculature after focal cerebral ischemia in adult rat. Ptn gene expression remains upregulated in these same cells and sites 7 and 14 d after ischemic injury. However, expression of the Ptn gene is significantly decreased in cortical neurons 6 and 24 hr after injury and is undetectable in degenerating neurons at day 3. Neurons in contralateral cortex continue to express Ptn in levels equal to control, uninjured brain. It is suggested that PTN may have a vital role in neovascular formation in postischemic brain and that postischemic brain is an important model in which to analyze sequential gene expression in developing neovasculature. In contrast, Ptn gene expression in injured neurons destined not to recover is strikingly reduced, and potentially its absence may contribute to the failure of the neuron to survive.

2/3,AB/30
DIALOG(R)File 155:MEDLINE(R)

09618039 98049437 PMID: 9389569
Differential expression and biological activity of the heparin-binding growth-associated molecule (HB-GAM) in lung cancer cell lines. Jager R; Noll K; Havemann K; Pfluger K H; Knabbe C; Rauvala H; Zugmaier G
Department of Hematology/Oncology, Philipps-Universitat, Marburg, Germany.
International journal of cancer. Journal international du cancer (UNITED STATES) Nov 14 1997, 73 (4) p537-43, ISSN 0020-7136 Journal Code: 0042124
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The growth of human lung cancer cells is regulated positively and negatively by a variety of growth factors through autocrine as well as paracrine mechanisms. In the present report, we studied the differential role and expression of a neuropolypeptide growth factor in 26 lung cancer cell lines. Expression of the heparin-binding growth-associated molecule (HB-GAM) in 12 small cell lung cancer (SCLC) cell lines was compared to that in 14 non-small cell lung cancer (NSCLC) cell lines. HB-GAM mRNA was expressed in 9 of 12 SCLC and 3 of 14 NSCLC cell lines as determined by RT-PCR analyses. Normal human bronchial epithelial cells were used as negative controls. All cell lines

which expressed HB-GAM mRNA produced HB-GAM protein as well. Western blot analysis showed that the tumor cells secreted HB-GAM into the media. HB-GAM, purified from lung cancer cell lines, exerted biological activity on fibroblasts, endothelial cells and SW13 cells as determined by thymidine incorporation and soft agar cloning assays. In addition, the biological activity of HB-GAM was blocked by a specific antibody in a dose-dependent way. Our findings suggest that HB-GAM may serve as a marker for SCLC cell lines and that it may function as a paracrine growth factor in human lung cancer. HB-GAM may be a further member of the network of growth factors involved in proliferation, *angiogenesis* and metastasis of lung tumors.

2/3,AB/31
DIALOG(R)File 155:MEDLINE(R)

09531805 97429261 PMID: 9283611
Cellular distribution of the *angiogenic* factor heparin affin regulatory peptide (HARP) mRNA and protein in the human mammary gland. Ledoux D; Caruelle D; Sabourin J C; Liu J; Crepin M; Barritault D; Courty J
Laboratoire de Recherche sur la Croissance Cellulaire, la Reparation et la Regeneration Tissulaires, Unite CNRS Associee 1813, Universite Paris XII, Creteil, France.
journal of histochemistry and cytochemistry : official journal of the Histochemistry Society (UNITED STATES) Sep 1997, 45 (9) p1239-45, ISSN 0022-1554 Journal Code: 9815334
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The heparin affin regulatory peptide (HARP) growth factor, also known as *pleiotrophin* , is a developmentally regulated protein that displays biological functions during cell growth and differentiation. To study the physiological role of this protein, we investigated the cellular distribution of HARP mRNA and protein in the resting human mammary gland. In situ hybridization histochemistry revealed that HARP mRNA was localized in alveolar myoepithelial cells, whereas alveolar epithelial cells were negative. In the stroma, HARP mRNA was localized in endothelial cells and smooth muscle cells of blood vessels. Interestingly, HARP protein and mRNA were not always co-localized. HARP protein immunocytochemistry staining was observed in an area including both alveolar myoepithelial and epithelial cells, although epithelial cells do not express HARP transcript. In contrast, the distribution of HARP protein is parallel to that of HARP mRNA in endothelial and vascular smooth muscle cells. In the light of these results, the putative role of HARP in controlling the proliferation and/or differentiation of the different mammary cell types is proposed and discussed.

2/3,AB/32
DIALOG(R)File 155:MEDLINE(R)

09471943 97382295 PMID: 9235965
Signal transduction pathways involved in the mitogenic activity of *pleiotrophin* . Implication of mitogen-activated protein kinase and phosphoinositide 3-kinase pathways.
Souttou B; Ahmad S; Riegel A T; Wellstein A
Lombardi Cancer Center and Department of Pharmacology, Georgetown University, Washington, DC 20007, USA.
Journal of biological chemistry (UNITED STATES) Aug 1 1997, 272 (31) p19588-93, ISSN 0021-9258 Journal Code: 2985121R
Contract/Grant No.: CA58185; CA; NCI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Pleiotrophin (PTN) is a developmentally regulated protein which exhibits neurite-outgrowth, mitogenic, and *angiogenic* properties. It has also been shown to be involved in tumor growth and metastasis. Here we used primary BEL (bovine epithelial lens) cells to investigate the signal transduction pathways involved in the mitogenic activity of recombinant PTN. PTN was purified from conditioned media of SW-13 cells transfected with the human PTN cDNA. We show that inhibitors of tyrosine kinase, mitogen-activated protein kinase, or phosphoinositide (PI) 3-kinase inhibit DNA synthesis stimulated by PTN. Analysis of tyrosine-phosphorylated proteins following PTN stimulation showed phosphorylation of two novel 190- and 215-kDa proteins in addition to SHC, ERK1, and ERK2. A mobility shift of phosphorylated ERK1 and ERK2 was detected with a panERK antibody confirming the phosphorylation of the two ERKs. Furthermore, in vitro immunocomplex kinase assay with Akt1, a natural substrate of PI 3-kinase, showed an activation of the kinase following PTN stimulation and a reversal by the PI 3-kinase inhibitor wortmannin. We conclude that the mitogenic activity of PTN is dependent on tyrosine kinase activation and utilizes the mitogen-activated protein kinase and the PI 3-kinase pathways to transduce a mitogenic signal.

2/3,AB/33
DIALOG(R)File 155:MEDLINE(R)

09444654 97347469 PMID: 9201975
Human breast cancer growth inhibited in vivo by a dominant negative *pleiotrophin* mutant.
Zhang N; Zhong R; Wang Z Y; Deuel T F

Division of Growth Regulation, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts 02215, USA.

Journal of biological chemistry (UNITED STATES) Jul 4 1997, 272 (27) p16733-6, ISSN 0021-9258 Journal Code: 2985121R

Contract/Grant No.: CA49712-06; CA; NCI; CA66029; CA; NCI; HL14147; HL; NHLBI

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Pleiotrophin (PTN) is a recently described 18- kDa heparin binding growth/differentiation factor. It also is a proto-oncogene; cells transformed by the Ptn gene form highly *angiogenic* tumors when implanted into the nude mouse. PTN may be an important regulator of transformation in other tumors, because constitutively high levels of expression of the *pleiotrophin* (Ptn) gene are found in human breast cancer and other malignant cell lines, and its levels of expression are high in many human tumor specimens. To determine whether PTN is an important regulator of the malignant phenotype of human breast cancer cells, we constructed a mutant cDNA to encode a truncated PTN designed to heterodimerize with the product of the endogenous Ptn gene during processing. The mutant gene product blocked transformation of NIH 3T3 cells by the wild type (wt) Ptn gene product. The mutant Ptn cDNA was then introduced into human breast cancer MDA-MB-231 cells, and clonal lines that stably express the mutant Ptn cDNA were selected. The truncated PTN was shown to form heterodimers with the endogenous Ptn gene product in these cells. Furthermore, the MDA-MB-231 cells that express the mutant Ptn gene were no longer transformed; they failed to form plaques or colonies in soft agar and were unable to form tumors in the athymic nude mouse. The results establish an important role of PTN in the dysregulated growth of human breast cancer cells and suggest that constitutive expression of PTN may be essential to the malignant phenotype of human breast cancers in vivo.

2/3,AB/34
DIALOG(R)File 155:MEDLINE(R)

09376391 97280700 PMID: 9135027

An *angiogenic* role for the neurokines midkine and *pleiotrophin* in tumorigenesis.

Choudhuri R; Zhang H T; Donnini S; Ziche M; Bicknell R

Molecular Angiogenesis Group, Imperial Cancer Research Fund, Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, United Kingdom.

Cancer research (UNITED STATES) May 1 1997, 57 (9) p1814-9, ISSN 0008-5472 Journal Code: 2984705R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Recent analysis of bladder tumors has correlated expression of the neurokine midkine (MK) with poor patient survival. To examine a role for MK and the related *pleiotrophin* (PTN) in tumorigenesis, they were overexpressed in MCF-7 breast carcinoma cells. Expression had no effect on in vitro growth but conferred a growth advantage in vivo. Enhanced tumor growth correlated with increased vascular density and endothelial proliferation, implicating an *angiogenic* role for MK and PTN. *Angiogenic* activity of MK and PTN was confirmed in the rabbit corneal assay. Our data therefore identify two novel targets for antiangiogenic drug development.

2/3,AB/35
DIALOG(R)File 155:MEDLINE(R)

09297456 97193617 PMID: 9041202

Expression of the *angiogenic* factors vascular endothelial cell growth factor, acidic and basic fibroblast growth factor, tumor growth factor beta-1, platelet-derived endothelial cell growth factor, placenta growth factor, and *pleiotrophin* in human primary breast cancer and its relation to *angiogenesis*.

Relf M; LeJeune S; Scott P A; Fox S; Smith K; Leek R; Moghaddam A; Whitehouse R; Bicknell R; Harris A L

Imperial Cancer Research Fund, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, England.

Cancer research (UNITED STATES) Mar 1 1997, 57 (5) p963-9, ISSN 0008-5472 Journal Code: 2984705R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Angiogenesis is a significant prognostic factor in breast cancer, but the factors that control *angiogenesis* in vivo are not well defined. Multiple *angiogenic* polypeptides are known, and we have determined the expression of seven of these in primary human breast cancers; the relationship of expression to estrogen receptor and vascular density was also examined. Vascular endothelial growth factor (VEGF) and its four isoforms (121, 165, 189, and 206 amino acids), transforming growth factor (TGF)-beta1, *pleiotrophin*, acidic and basic fibroblast growth factor (FGF), placental growth factor, and thymidine phosphorylase (platelet-derived endothelial cell growth factor) were quantitated by RNase protection analysis. beta-FGF was also measured by ELISA. The estrogen receptor (ER), epidermal growth factor receptor, and vascular density were analyzed in 64 primary breast cancers. All tumors expressed at least six different vascular growth factors. VEGF was most abundant, and the transcript

for the 121-amino acid form predominated. Other *angiogenic* factors expressed at high levels were thymidine phosphorylase and TGF-beta1. Expression of most of the *angiogenic* factors did not correlate with that of ER or vascular density. However, thymidine phosphorylase did, with a correlation coefficient of 0.3 (P = 0.03). There were significant associations of *pleiotrophin* with acidic FGF expression (P = 0.001) and TGF-beta with platelet-derived endothelial cell growth factor expression (P = 0.001). Thus, *angiogenesis* may involve a coordinate regulation of some vascular growth factors. High VEGF expression correlated with poor prognosis in univariate analysis (P = 0.03), as did ER and epidermal growth factor receptor expression. Basic FGF was also assessed by ELISA and was more highly expressed in tumors than normal breast tissues (median, 346 microg/ml cytosol; range, 54-1323 versus median, 149; range, 32-509; P = 0.01). Implications for therapy are that broad spectrum agents that block features common to these factors may be useful (e.g., antagonism of heparin-binding activity agents), because so many *angiogenic* factors are expressed. Inhibiting endothelial migration or agents directly toxic to endothelium would be of value in a combined approach to therapy.

2/3,AB/36
DIALOG(R)File 155:MEDLINE(R)

09224778 97121462 PMID: 8962128
Human trophoblast and choriocarcinoma expression of the growth factor *pleiotrophin* attributable to germ-line insertion of an endogenous retrovirus.
Schulte A M; Lai S; Kurtz A; Czubayko F; Riegel A T; Wellstein A Lombardi Cancer Center, Georgetown University, Washington, DC 20007, USA. Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Dec 10 1996, 93 (25) p14759-64, ISSN 0027-8424 Journal Code: 7505876
Contract/Grant No.: CA58185; CA; NCI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Retroviral elements are found in abundance throughout the human genome but only rarely have alterations of endogenous genes by retroviral insertions been described. Herein we report that a human endogenous retrovirus (HERV) type C is inserted in the human growth factor gene *pleiotrophin* (PTN) between the 5' untranslated and the coding region. This insert in the human genome expands the region relative to the murine gene. Studies with promoter-reporter constructs show that the HERV insert in the human PTN gene generates an additional promoter with trophoblast-specific activity. Due to this promoter function, fusion transcripts between HERV and the open reading frame of PTN (HERV-PTN) were detected in all normal human trophoblast cell cultures as early as 9 weeks after gestation (n = 7) and in all term placenta tissues (n = 5) but not in other normal adult tissues. Furthermore, only trophoblast-derived choriocarcinoma cell lines expressed HERV-PTN mRNA whereas tumor cell lines derived from the embryoblast (teratocarcinoma) or from other lineages failed to do so. We investigated the significance of HERV-PTN mRNA in a choriocarcinoma model by targeting this transcript with ribozymes and found that the depletion of HERV-PTN mRNA prevents human choriocarcinoma growth, invasion, and *angiogenesis* in mice. This suggests that the tissue-specific expression of PTN due to the HERV insertion in the human genome supports the highly aggressive growth of human choriocarcinoma and possibly of the human trophoblast.

2/3,AB/37
DIALOG(R)File 155:MEDLINE(R)

09224777 97121461 PMID: 8962127
Melanoma *angiogenesis* and metastasis modulated by ribozyme targeting of the secreted growth factor *pleiotrophin*. Czubayko F; Schulte A M; Berchem G J; Wellstein A
Lombardi Cancer Center, Georgetown University, Washington, DC 20007, USA. Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Dec 10 1996, 93 (25) p14753-8, ISSN 0027-8424 Journal Code: 7505876
Contract/Grant No.: CA58185; CA; NCI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Clinical and experimental evidence suggests that spreading of malignant cells from a localized tumor (metastasis) is directly related to the number of microvessels in the primary tumor. This tumor *angiogenesis* is thought to be mediated by tumor-cell-derived growth factors. However, most tumor cells express a multitude of candidate *angiogenesis* factors and it is difficult to decipher which of these are rate-limiting factors in vivo. Herein we use ribozyme targeting of *pleiotrophin* (PTN) in metastatic human melanoma cells to assess the significance of this secreted growth factor for *angiogenesis* and metastasis. As a model we used human melanoma cells (1205LU) that express high levels of PTN and metastasize from subcutaneous tumors to the lungs of experimental animals. In these melanoma cells, we reduced PTN mRNA and growth factor activity by transfection with PTN-targeted ribozymes and generated cell lines expressing different levels of PTN.

We found that the reduction of PTN does not affect growth of the melanoma cells in vitro. In nude mice, however, tumor growth and *angiogenesis* were decreased in parallel with the reduced PTN levels and apoptosis in the tumors was increased. Concomitantly, the metastatic spread of the tumors from the subcutaneous site to the lungs was prevented. These studies support a direct link between tumor *angiogenesis* and metastasis through a secreted growth factor and identify PTN as a candidate factor that may be rate-limiting for human melanoma metastasis.

2/3,AB/38
DIALOG(R)File 155:MEDLINE(R)

09195189 97099501 PMID: 8944070
Midkine in the progression of rat N-nitroso-N-methylurea-induced mammary tumors.
Chen Y; McKenzie K E; Aldaz C M; Sukumar S
Breast Cancer Program, Johns Hopkins Oncology Center, Baltimore, Maryland 21205, USA.
Molecular carcinogenesis (UNITED STATES) Nov 1996, 17 (3) p112-6, ISSN 0899-1987 Journal Code: 8811105
Contract/Grant No.: 1RO1 CA 57993; CA; NCI; CA 59967; CA; NCI Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Recent studies have implicated a role for midkine (MK) in cancer progression. This is based upon its structural homology with *pleiotrophin*, an *angiogenic* growth factor, and its ability to enhance fibrinolytic activity of bovine endothelial cells. To investigate whether MK plays a role in breast cancer, we examined MK mRNA expression in N-nitroso-N-methylurea-induced rat mammary tumors at various stages of tumor progression, including hormone independence and distant metastasis. Well-differentiated mammary adenocarcinomas showed levels of MK comparable to those of normal mammary gland. A 10- to 20-fold reduction in MK mRNA levels was observed in mammary tumors that had progressed to hormone independence and metastasis. The data suggest that loss of MK expression correlates with breast tumor progression. Treatment of rat mammary tumor cell lines with retinoic acid increased MK expression, decreased proliferation, and markedly reduced colony-forming efficiency in agar. This raises the possibility that agents that upregulate MK could have potential in prevention and therapy by causing breast cells to terminally differentiate.

2/3,AB/39
DIALOG(R)File 155:MEDLINE(R)

09186607 97067374 PMID: 8910787
Localization of *pleiotrophin* and its mRNA in subpopulations of neurons and their corresponding axonal tracts suggests important roles in neural-glial interactions during development and in maturity.
Silos-Santiago I; Yeh H J; Gurrieri M A; Guillerman R P; Li Y S; Wolf J; Snider W; Deuel T F
Department of Neurology and Neurological Surgery, Washington University School of Medicine, St. Louis, Missouri 63110, USA.
Journal of neurobiology (UNITED STATES) Nov 1996, 31 (3) p283-96, ISSN 0022-3034 Journal Code: 0213640
Contract/Grant No.: CA49712; CA; NCI; HL14147; HL; NHLBI; HL31102; HL; NHLBI; +
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Trophic factors are being increasingly recognized as important contributors to growth, differentiation, and maintenance of viability within the mammalian nervous system during development. *Pleiotrophin* (PTN) is a secreted 18-kDa heparin binding protein that stimulates mitogenesis and *angiogenesis* and neurite and glial process outgrowth guidance activities in vitro. We localized the sites and time course of expression of the Ptn gene and its protein product in developing and adult mouse nervous system. Expression of the Ptn gene was first observed at embryo day 8.5 (E8.5). At E12.5, transcripts of the Ptn gene were localized in developing neuroepithelium at sites of active cell division in the spinal cord and brain. At E15.5, transcripts were found in the somata of some but not all neurons and glia whereas in the adult its pattern of expression was nearly exclusively restricted to the brain. The PTN protein was found almost entirely in association with the axonal tracts during development and in adults. Furthermore, as opposed to the finding of PTN in both central and peripheral nervous systems during development, PTN was not expressed beyond the exit where axonal tracts become the peripheral nervous system in adults. At all sites and times examined, the somata that contained Ptn transcripts corresponded with the axonal tracts that contained the PTN protein. The results establish that Ptn is expressed in early development at sites of active mitogenesis in developing neuroepithelium and later in both glial cells and neurons at sites of neuronal and glial process formation in developing axonal tracts. The findings establish a correspondence in the localization of PTN within the nervous system at sites of normal developmental processes that correlate with the functional activities of PTN previously described in vitro.

2/3,AB/40
DIALOG(R)File 155:MEDLINE(R)

09046884 96422510 PMID: 8825127
Breast cancer *angiogenesis* --new approaches to therapy via antiangiogenesis, hypoxic activated drugs, and vascular targeting. Harris A L; Zhang H; Moghaddam A; Fox S; Scott P; Pattison A; Gatter K; Stratford I; Bicknell R
Institute of Molecular Medicine, John Radcliffe Hospital, Oxford, U.K. Breast cancer research and treatment (NETHERLANDS) 1996, 38 (1) p97-108, ISSN 0167-6806 Journal Code: 8111104
Document type: Journal Article; Review; Review, Tutorial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Several groups have shown that quantitation of tumor *angiogenesis* by counting blood vessels in primary breast cancer gives an independent assessment of prognosis. Poor prognosis is associated with high blood vessel counts. We have shown that the rate of cell division in endothelial cells is much higher in breast tumours than in normal breast. Breast cancer cell lines and primary human breast tumours express a wide range of vascular growth factors, including VEGF, placenta growth factor, *pleiotrophin* , TGF beta 1, acidic and basic FGF, and platelet-derived endothelial cell growth factor. Inhibiting *angiogenesis* by blocking vascular growth factors would be difficult with highly specific agents, but drugs with a broader spectrum of antagonism may be effective. We have developed several suramin analogues which are less toxic than suramin in vivo but more potent in inhibiting *angiogenesis*, and these have been developed for Phase I. A combination of anti-*angiogenesis* agents with drugs activated by hypoxia may also be useful, because anti-*angiogenesis* alone may not kill cells, whereas activation of hypoxic drugs could synergize. New endpoints may be necessary because inhibition of new blood vessel formation may not cause tumour regression. Thus, the endpoint of stable disease and biochemical assessment of inhibition of *angiogenesis* may be much more important in therapeutic studies and for drug development in the future. The prognostic importance of *angiogenesis* suggests that this should be a major new therapeutic target.

2/3,AB/41
DIALOG(R)File 155:MEDLINE(R)

09012678 96384191 PMID: 8792088
Pleiotrophin and midkine in normal development and tumor biology. Kurtz A; Schulte A M; Wellstein A
Lombardi Cancer Center, Georgetown University, Washington, DC 20007, USA. Critical reviews in oncogenesis (UNITED STATES) 1995, 6 (2) p151-77, ISSN 0893-9675 Journal Code: 8914610
Document type: Journal Article; Review; Review, Academic Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Pleiotrophin (PTN) and midkine (MK) are members of a family of developmentally regulated, secreted heparin-binding proteins. The proteins are structural homologs, and are highly conserved among species. Although no homology has been detected with other heparin-binding growth factors, their functional similarity to members of the fibroblast growth factor (FGF) family is remarkable. PTN and MK are expressed during embryogenesis, showing an expression pattern that suggests functions in neurogenesis, cell migration, secondary organogenetic induction, and mesoderm-epithelial interaction. The widespread downregulation of PTN and MK in the adult human is reverted in a number of cancers, in which polypeptides are able to act as both transforming growth factors and promoters of *angiogenesis*. Elucidating the molecular mechanisms of PTN and MK action could lead not only to a deeper understanding of developmental processes, but also to the ultimate identification of targets for tumor therapy.

2/3,AB/42
DIALOG(R)File 155:MEDLINE(R)

08707595 96049800 PMID: 8534864
Molecular and pharmacologic targeting of *angiogenesis* factors--the example of *pleiotrophin*.
Czubayko F; Schulte A M; Missner S C; Hsieh S S; Colley K J; Wellstein A Lombardi Cancer Center, Georgetown University, Washington D.C. 20007, USA.
Breast cancer research and treatment (NETHERLANDS) 1995, 36 (2) p157-68, ISSN 0167-6806 Journal Code: 8111104
Document type: Journal Article; Review; Review, Tutorial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Polypeptide growth factors contribute to the development and maintenance of normal tissues and are essential for the growth and metastasis of solid tumors. During tumor progression these factors function as autocrine stimulators of tumor cells and/or serve to recruit stromal tissue and blood supply to the expanding tumor. In particular, tumor-induced *angiogenesis* appears to be significant not only for local tumor growth but also for metastasis to distant organ sites. We purified several years ago the heparin-binding growth factor *pleiotrophin* (PTN) from the supernatants of human breast cancer cells and demonstrated that PTN can serve as an *angiogenesis* factor. We found the gene expressed in a number of human tumor cell lines as well as in human tumor tissues. Here we present different approaches to inhibit production and function of this growth factor. Finally we discuss how the experience from this growth factor

can be applied to improve our understanding of the role of other factors thought to contribute to tumor *angiogenesis*.

2/3,AB/43
DIALOG(R)File 155:MEDLINE(R)

08554061 95310386 PMID: 7790396
Effect of heparin on bovine epithelial lens cell proliferation induced by heparin affin regulatory peptide.
Delbe J; Vacherot F; Laaroubi K; Barritault D; Courty J
Laboratoire de Recherche sur la Croissance Cellulaire, la Reparation et la Regeneration Tissulaires (CRRET), URA CNRS, Creteil, France. Journal of cellular physiology (UNITED STATES) Jul 1995, 164 (1) p47-54, ISSN 0021-9541 Journal Code: 0050222
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
HARP (heparin affin regulatory peptide) is an 18 kDa heparin binding protein, also known as HB-GAM or *pleiotrophin* (PTN) which has been primarily isolated from brain and uterus, and displays neurite outgrowth, *angiogenic* and mitogenic activities. Previously, we have expressed the human cDNA encoding human HARP in NIH 3T3 cells. Purified recombinant HARP displayed mitogenic activity for endothelial cells. Its NH2-terminal sequence indicates that the HARP molecule possesses a three amino acid extension from the signal peptide more than the NH2-terminal described. For HB-GAM or PTN, these three amino acids may be essential for the stability and the mitogenic activity of this growth factor. In an attempt to further study the mode of action of this growth factor, we have investigated the mitogenic effect of HARP on various cell types. In contrast to FGF-2, HARP failed to induce stimulation of DNA synthesis on a CCL39 cell line. However, we found that in quiescent bovine epithelial lens (BEL) cells, the stimulation of DNA synthesis induced by HARP is dose-dependent (EC50: 2.5 ng/ml) and maximal stimulation is as potent as that induced by FGF-2 (EC50: 25 pg/ml). Interestingly, when BEL cells were allowed to quiesce in the presence of serum, the stimulation induced by HARP is considerably less potent. In this highly responsive cell system, heparin could potentiate the mitogenic activity of HARP at very low doses (0.1-1 microgram/ml) and inhibit this activity at concentrations of 10 micrograms/ml. In contrast to its protective effect on FGF-1 and -2, heparin was unable to preserve HARP from tryptic and chymotryptic degradations.

2/3,AB/44
DIALOG(R)File 155:MEDLINE(R)

08501453 95256346 PMID: 7537745
The isolation and long-term culture of normal human endometrial epithelium and stroma. Expression of mRNAs for *angiogenic* polypeptides basally and on oestrogen and progesterone challenges. Zhang L; Rees M C; Bicknell R
Institute of Molecular Medicine, University of Oxford, UK. Journal of cell science (ENGLAND) Jan 1995, 108 (Pt 1) p323-31, ISSN 0021-9533 Journal Code: 0052457
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
A highly reproducible and technically straightforward technique for the isolation and long-term culture of normal human endometrial epithelial cells is described. The essential conditions for long-term culture are that the cells be seeded onto a gelatin matrix and that 'endothelial cell growth supplement' be present in the culture medium. Normal endometrial epithelial cells express cytokeratins and oestrogen receptors. They may be passaged five to six times without change in properties. Growth of normal endometrial epithelial cells was stimulated by 17-beta-oestradiol and epidermal growth factor. Expression of the mRNA coding for seven polypeptide *angiogenic* factors, by normal endometrial epithelial, stromal and three endometrial carcinoma lines, was examined. The endometrial epithelial and stromal cells express mRNA for the polypeptide *angiogenic* factors, basic fibroblast growth factor, vascular endothelial cell growth factor, transforming growth factor-beta 1 and *pleiotrophin*, as well as the cytokine midkine. Expression of the mRNA for both vascular endothelial growth factor and midkine by normal endometrial epithelial cells showed a 2-fold increase on treatment with a physiological dose of 17-beta-oestradiol (10(-10) M) while, in contrast, the mRNA of transforming growth factor-beta 1 decreased 4-fold on treatment with 17-beta-oestradiol (10(-10) M) and was abolished by exposure to progesterone (5 x 10(-9) M). Expression of the mRNAs for *angiogenic* polypeptides by the endometrial carcinoma lines was more restricted.

2/3,AB/45
DIALOG(R)File 155:MEDLINE(R)

08417092 95186809 PMID: 7533562
The potential role of the heparin-binding growth factor *pleiotrophin* in breast cancer.
Riegel A T; Wellstein A
Vincent T. Lombardi Cancer Center, Georgetown University, Washington, DC 20007.
Breast cancer research and treatment (NETHERLANDS) 1994, 31 (2-3) p309-14, ISSN 0167-6806 Journal Code: 8111104
Document type: Journal Article; Review; Review, Tutorial
Languages: ENGLISH
Main Citation Owner: NLM

Record type: Completed
We propose that the secreted protein *pleiotrophin* (PTN) is a major factor in the malignant progression of breast cancer. This hypothesis is based on the growth-stimulatory effects of PTN on cells in vitro and in vivo and on its high levels of expression in 60% of tumor samples from breast cancer patients. The stimulation of proliferation and tube formation of endothelial cells by PTN suggests that it can serve as an *angiogenesis* factor during tumor growth. We hypothesize that PTN has the potential to support growth of breast cancer at its primary site and to enhance the ability of tumor cells to metastasize. Furthermore, we suggest that specific endocrine signals interact to regulate the expression of PTN in vitro and in vivo. Finally, we propose that understanding the functions of PTN and its hormonal regulation can lead to the development of novel therapeutic strategies for breast cancer.

2/3,AB/46
DIALOG(R)File 155:MEDLINE(R)

08210988 94347392 PMID: 7520717
Mitogenic and in vitro *angiogenic* activity of human recombinant heparin affin regulatory peptide.
Laaroubi K; Delbe J; Vacherot F; Desgranges P; Tardieu M; Jaye M; Barritault D; Courty J
Laboratorie de Recherche sur la Croissance Cellulaire, Universite Paris Val de Marne, Creteil, France.
Growth factors (Chur, Switzerland) (SWITZERLAND) 1994, 10 (2) p89-98 , ISSN 0897-7194 Journal Code: 9000468
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
We have previously described the purification of a heparin binding growth factor from adult bovine brain named heparin affin regulatory peptide (HARP), which was identical to an uterus derived growth factor named *pleiotrophin* and to a developmentally regulated neurite promoting factor named heparin-binding growth associated molecule. However, for yet unclear reasons, the mitogenic activity of this purified polypeptide following isolation from animal tissue extracts is a subject of controversy, due to conflicting and irreproducible data when produced by recombinant DNA technologies in E. coli or insect cells. The purified protein was inactive in mitogenic assays but the natural molecule was active in assay of neurite outgrowth. In order to clarify these conflicting results and to obtain a recombinant protein free from other contaminating heparin-binding growth factors, we have cloned human cDNA encoding human HARP, engineered its expression in NIH 3T3 cells and characterised the resulting recombinant polypeptide. Purified recombinant HARP displayed mitogenic activity for capillary endothelial cells with half-maximal stimulation at approximately 1 ng/ml (55 pM) and induced *angiogenesis* in an in vitro model. Interestingly, while the NH2 terminal sequence of tissue purified HARP was NH2-GKKEKPEKK, the NH2 terminal sequence of the biologically active recombinant protein was NH2-AEAGKKEKPEKK, corresponding to a three amino acid extended form.

2/3,AB/47
DIALOG(R)File 155:MEDLINE(R)

08205022 94340507 PMID: 7520350
Midkine and *pleiotrophin* expression in normal and malignant breast tissue.
Garver R I; Radford D M; Donis-Keller H; Wick M R; Milner P G Department of Medicine, University of Alabama Birmingham School of Medicine.
Cancer (UNITED STATES) Sep 1 1994, 74 (5) p1584-90, ISSN 0008-543X Journal Code: 0374236
Contract/Grant No.: HG00201; HG; NHGRI; HL47380; HL; NHLBI Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
BACKGROUND. Some growth factors may promote tumor growth by affecting tumor *angiogenesis*. The *angiogenic* growth factor, *pleiotrophin* , was demonstrated previously in human breast carcinoma tissues; however, the pattern of *pleiotrophin* expression in normal breast tissues has not been established. METHODS. The expression of *pleiotrophin* and the related growth factor, midkine, was examined by polymerase chain reaction amplification of reverse transcriptase copies of RNA transcripts (RT-PCR) from freshly resected normal and malignant human breast tissues. Northern blot analysis of midkine expression was performed on a limited number of the specimens and on human and canine breast carcinoma cell lines. Clinicopathologic variables from the breast cancer patients were examined in relation to the growth factor expression patterns. RESULTS. The majority of both malignant and normal breast tissues expressed *pleiotrophin*. In contrast, midkine was expressed frequently in the malignant breast tissues but in only one of the normal specimens. Northern blot analysis of the breast carcinoma cells lines showed that they commonly expressed midkine transcripts. The only correlation of the growth factor expression patterns with the other clinical variables was the finding that the three midkine-negative breast carcinoma specimens also had low estrogen receptor levels. CONCLUSIONS. By this analysis, the expression of *pleiotrophin* was equivalent in both malignant and normal human breast tissues. Midkine, on the other hand, exhibited increased expression in the breast carcinomas but showed much

lower expression in the normal breast tissue. Although the cellular source of the midkine expression was not determined by the RT-PCR assay, the Northern blot analysis showed that isolated populations of breast cancer cells commonly express this growth factor. This is the first example of a tissue simultaneously expressing high amounts of both *pleiotrophin* and midkine, a finding of unclear pathophysiologic significance.

2/3,AB/48
DIALOG(R)File 155:MEDLINE(R)

07895980 94031070 PMID: 8217186
Reciprocal expression of *pleiotrophin* and midkine in normal versus malignant lung tissues.
Garver R I; Chan C S; Milner P G
Department of Medicine, UAB School of Medicine.
American journal of respiratory cell and molecular biology (UNITED STATES) Nov 1993, 9 (5) p463-6, ISSN 1044-1549 Journal Code: 8917225 Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Abundant evidence suggests that growth factors are important mediators of non-small cell lung cancer (NSCLC) growth. Although multiple growth factors have been found to be produced by NSCLC tissues, little is known about possible differences in growth factor expression between malignant and adjacent normal lung tissues. Variation in growth factor expression between normal and malignant lung tissues could be potentially useful diagnostically and therapeutically. In studies reported here, the expression of the *angiogenic* growth factor *pleiotrophin* (PTN) and homolog midkine (MK) was assessed in resected normal and malignant lung tissues. Primers specific for the two growth factors were used to amplify reverse transcriptase-produced DNA copies of RNA transcripts harvested from the tissues. This analysis revealed that all normal lung tissues examined (n = 17) expressed PTN but only two expressed MK. Conversely, all of the resected lung cancers (n = 20) expressed MK but only one expressed PTN. These results demonstrated a striking reciprocal expression pattern of MK and PTN in normal versus malignant lung tissue.

2/3,AB/49
DIALOG(R)File 155:MEDLINE(R)

07574229 93100306 PMID: 1464602
Pleiotrophin stimulates fibroblasts and endothelial and epithelial cells and is expressed in human cancer.
Fang W; Hartmann N; Chow D T; Riegel A T; Wellstein A
V.T. Lombardi Cancer Center, Georgetown University, Washington, D.C. 20007.
Journal of biological chemistry (UNITED STATES) Dec 25 1992, 267 (36) p25889-97, ISSN 0021-9258 Journal Code: 2985121R
Contract/Grant No.: U01 CA51908; CA; NCI
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
Previously we reported the purification of the heparin-binding growth factor *pleiotrophin* (PTN) from supernatants of the human breast cancer cell line MDA-MB-231. To investigate further the biological activities of PTN and its potential role in cancer, we cloned a PTN cDNA and expressed the gene in a human kidney and in a human adrenal carcinoma cell line (SW-13). The supernatants harvested from cells transfected with PTN contained a heparin-binding specific protein of an apparent molecular mass of 18 kDa. These supernatants stimulated the proliferation of endothelial cells as well as the anchorage-independent growth of SW-13 cells and of normal rat kidney fibroblasts. Furthermore, SW-13 cells transfected with PTN acquired autonomous growth in soft agar and were tumorigenic in athymic nude mice. In contrast to these results with PTN from human cells, PTN obtained from insect cells (Sf9) using recombinant baculovirus as a vector was biologically inactive. We detected high levels of PTN mRNA in 16 of 27 primary human breast cancer samples (62%) as well as in 8 of 8 carcinogen-induced rat mammary tumors. Furthermore, 9 of 34 human tumor cell lines of different origin showed detectable PTN mRNA. We conclude that PTN may function as a tumor growth and *angiogenesis* factor in addition to its role during embryonic development. ? s fusion()protien
97237 FUSION
41 PROTIEN
S4 0 FUSION()PROTIEN
? s fusion()protein
97237 FUSION
1088158 PROTEIN
S5 13969 FUSION()PROTEIN
? ds

Set Items Description
S1 209 PLEIOTROPHIN
S2 49 S1 AND ANGIOGEN?
S3 9 S2 AND PY=2002
S4 0 FUSION()PROTIEN
S5 13969 FUSION()PROTEIN
? s s5 and s1
13969 S5
209 S1
S6 1 S5 AND S1
? t s6/3,ab/

6/3,AB/1
DIALOG(R)File 155:MEDLINE(R)

07875528 94013246 PMID: 8408430
Structural characterisation of native and recombinant forms of the neurotrophic cytokine MK.
Fabri L; Maruta H; Muramatsu H; Muramatsu T; Simpson R J; Burgess A W; Nice E C
Melbourne Tumour Biology Branch, Ludwig Institute for Cancer Research (Melbourne Branch), Royal Melbourne Hospital, Victoria, Australia. Journal of chromatography (NETHERLANDS) Aug 27 1993, 646 (1) p213-25 , ISSN 0021-9673 Journal Code: 0427043
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed
The retinoic acid (RA)-inducible midkine (MK) gene encodes a heparin-binding protein which can induce neurite outgrowth in cultured mammalian embryonic brain cells. This cytokine shares 65% amino acid sequence identity with another RA-inducible cytokine, pleiotropin (PTN). Both proteins contain 10 conserved cysteine residues, all of which appear to be disulphide linked. MK and PTN are also rich in lysine and arginine residues rendering them susceptible to proteolysis during purification, and making large-scale preparation of these molecules inherently difficult. Recombinant MK has been expressed as a *fusion* *protein* using a pGEX vector transfected into E. coli. To enable refolding of MK, the *fusion* *protein* was stored in solution at 4 degrees C for 14 days in the presence of dithiothreitol (DTT). Thrombin cleavage of the *fusion* *protein* , post storage, typically generated 5 mg of MK per litre of bacterial pellet. To establish the structural integrity of the recombinant product, we have analysed the refolding kinetics and compared the disulphide bond assignment of recombinant MK with that of native MK and native PTN. The synergistic use of micropreparative HPLC, to separate and recover in small eluant volumes enzymatically derived peptide fragments, with matrix assisted laser desorption mass spectrometry (MALD-MS) and N-terminal sequence analysis has allowed the unambiguous identification of the disulphide bonded fragments of native and recombinant MK. The disulphide bond assignment of MK is C12-C36, C20-C45, C27-C49, C59-C91 and C69-C101, and is equivalent to that of PTN.
? logoff
20oct02 10:37:45 User217743 Session D574.3
$5.38 1.682 DialUnits File155
$10.50 50 Type(s) in Format 4 (UDF)
$10.50 50 Types
$15.88 Estimated cost File155
$0.65 TELNET
$16.53 Estimated cost this search
$16.53 Estimated total session cost 1.906 DialUnits
Logoff: level 02.09.15 D 10:37:45